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HEALED SUBACUTE BACTERIAL ENDOCARDITIS

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Evidence for the statement that patients with subacute bacterial endocarditis may recover has hitherto been the fact that those with a typical clinical picture and from whose blood organisms are grown on cultures do survive and become free from symptoms and bacteria.[1] Libman [2] reported at least 3 per cent of recoveries in the usual type of the disease. He was also convinced that many more recoveries occur in persons with a mild form of the disease which is often overlooked.

Pathologic confirmation of healed subacute bacterial endocarditis is, however, largely indirect and inferential in nature. For example, Weiss and Rhodes [1] examined a group of hearts obtained at autopsy and selected 3 as being examples of completely healed subacute bacterial endocarditis. Others are described as suggesting a "healing" endocarditis. Hamman [3] similarly described 4 cases, 2 of which showed evidence of "almost complete healing of vegetations," while in the other 2 the cardiac lesions were of such a nature as to strongly imply a healed bacterial endocarditis rather than valves damaged by the rheumatic process.

It is not within the province of this report to enter into a discussion of what Libman terms "recurrent mild cases," or into the vagaries of the "bacteria-free" stage of the disease. Most observers are agreed that the clinical course of subacute bacterial endocarditis is often erratic. On the other hand, they do not share Libman's opinions and look on classic cases with gloomy prognosis.

With the newer methods of therapy, reports of clinical cures have become more frequent. However, the dearth of pathologic confirmation still persists. It is the purpose of this report to correct partially this disproportion between clinical observation and pathologic proof. The following 2 cases are examples of classic subacute bacterial endocarditis cured by the method of combined penicillin-heparin therapy.[4] Both patients later experienced congestive heart failure due to excessive valvular damage and finally came to autopsy.

CASE 1.—J. K. was a white woman, aged 33. The diagnosis had been established as subacute bacterial endocarditis, and the patient was transferred from the Hospital for Joint Diseases to the Jewish Hospital of Brooklyn on Nov. 9, 1943 for penicillin-heparin therapy. There was no past history of rheumatic fever or chorea. Her illness dated back seven months to a period marked by fever and malaise. Laboratory and clinical evidence prior to her admission included cultures of the blood positive for Streptococcus viridans on several occasions, crops of white-centered petechiae, a palpable spleen, moderate secondary anemia and cardiac murmurs. She had been seen in consultation by Dr. E. Libman, who concurred in the diagnosis. Prior to her admission to the Jewish Hospital she was treated with massive doses of sulfadiazine by the intravenous route. These were ineffectual. During the period of sulfonamide therapy sudden severe costovertebral pain on the right side, tenderness and hematuria developed, after which the kidney became palpable. The kidney gradually increased in size, reaching to the crest of the ilium. Sulfadiazine levels of the blood ranged from 36 to 98 mg. per hundred cubic centimeters without sterilizing the blood stream.

On admission to this hospital she appeared euphoric and chronically ill. There was a harsh, short, systolic murmur at the apex. There were numerous petechiae over the lower part of the abdomen, the left arm, the neck and the chest. The right kidney was tender and palpable at the iliac crest. The spleen was just palpable. Culture of the blood revealed one hundred and fifteen colonies of Str. viridans per plate. The sulfadiazine level of the blood, determined on the day of her admission, was 113 mg. per hundred cubic centimeters, and the urea nitrogen level was 150.4 mg. There was no oliguria. The sedimentation rate was 76 mm. per hour by the Westergren method.

On November 11 continuous penicillin-heparin therapy was begun. The patient received approximately 200,000 Oxford units of penicillin each day, dissolved in 1,000 to 1,500 cc. of isotonic solution of sodium chloride. She

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1. Weiss, S., and Rhodes, C. P.: Healing and Healed Vegetative (Subacute Bacterial) Endocarditis, New England J. Med. **199**:70, 1928.

2. Libman, E.: A Consideration of the Prognosis in Subacute Bacterial Endocarditis, Am. Heart J. **1**: 25, 1922; A Further Report on Recovery and Recurrence in Subacute Bacterial Endocarditis, Tr. A. Am. Physician **48**: 44, 1933.

3. Hamman, L.: Healed Bacterial Endocarditis, Ann. Int. Med. **11**:175, 1937.

4. Loewe, L.; Rosenblatt, P.; Greene, H. J., and Russell, M.: Combined Penicillin and Heparin Therapy of Subacute Bacterial Endocarditis, J. A. M. A. **124**: 144 (Jan. 15) 1944. Loewe, L.: The Louis Gross Memorial Lecture: The Combined Use of Penicillin and Heparin in the Treatment of Subacute Bacterial Endocarditis, Canad. M. A. J. **52**:1 (Jan.) 1945.

also received approximately 200 mg. of heparin (Liquaemin) daily, which was also incorporated in the isotonic solution. From a control level of twelve minutes, the coagulation time was maintained between thirty and sixty minutes throughout the course of therapy. Treatment was discontinued on November 25 after the patient had received a total of 2,740,000 Oxford units of penicillin and 2,500 mg. of heparin. Cultures of the blood taken on November 19 and 29 and on December 6 showed no growth. During this course of therapy, the kidney and spleen gradually receded in size and finally became impalpable. Because the temperature did not return to normal, the patient was suspected of being in the clinically active but bacteria-free stage of the disease. Culture of the blood taken on December 8 revealed three colonies of Str. viridans per plate, and, accordingly, penicillin-heparin therapy was again started, on December 9. She was given intravenous therapy continuously for thirty-one days until Jan. 8, 1944, receiving a total of 6,000,000 Oxford units of penicillin and 5,600 mg. of heparin during this period. Two cultures of blood taken during the course of treatment were sterile, as were ten others made at approximately weekly intervals after the treatment up to March 7. On January 12, the patient menstruated for the first time since October. Her temperature became normal about this date and remained so until her discharge on March 12. On March 7 the sedimentation rate was 25 mm. per hour. Prior to her discharge it was noted that the character of the cardiac sounds had changed. At the apex there was a short rumbling presystolic and a long, high-pitched diastolic murmur. The high-pitched diastolic murmur was loudest at the fourth intercostal space just to the left of the sternum and could be faintly heard at the aortic area as well. While the blood pressure on her admission was 120 systolic and 80 diastolic, on discharge the tension was 126 systolic and 40 diastolic. She was discharged with a diagnosis of healed subacute bacterial endocarditis and aortic insufficiency for convalescent care at home, despite the fact that she was in a state of subclinical cardiac failure.

Fig. 1 (case 1).—Mitral valve ring showing increased vascularity compatible with old rheumatic infection. Hematoxylin and eosin; × 75.

Subsequent Course.—Despite greatly restricted physical activity, administration of digitalis and limitation of her intake of fluids, the patient's compensation gradually decreased. The liver increased in size and tenderness, and she finally became dyspneic and orthopneic even with complete rest in bed.

Final Hospitalization.—The patient had to be admitted to the New Rochelle Hospital on April 19 because of severe congestive heart failure. Physical examination revealed the heart to be grossly enlarged to the right and left. The liver was tender and palpable below the um-

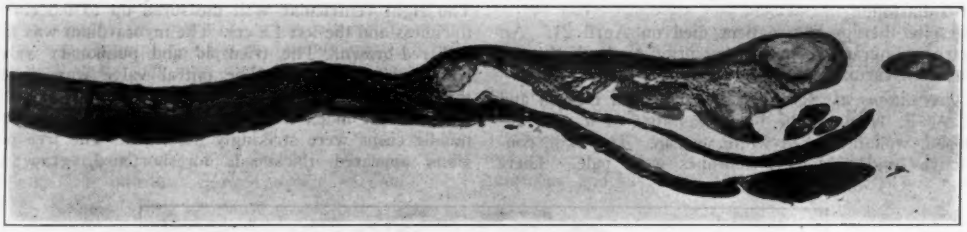

Fig. 2 (case 1).—Mitral valve leaflet with endothelialized nodules composed of loose fibrous connective tissue. These may represent healed, organized vegetations. Hematoxylin and eosin; × 11.

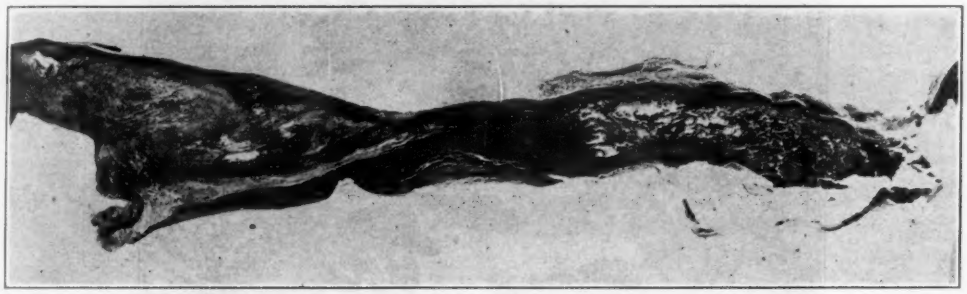

Fig. 3 (case 1).—Anterior aortic cusp; low power view of thickened, fragmented valve. Hematoxylin and eosin; × 11.

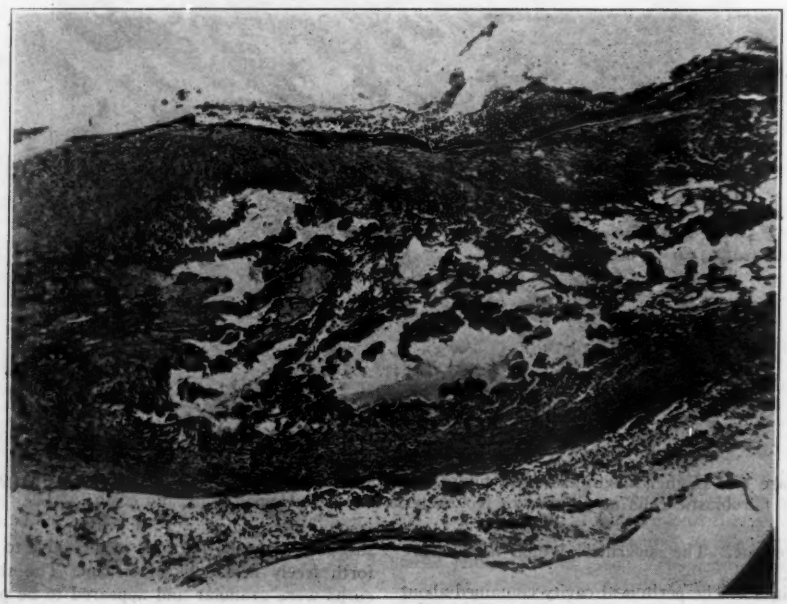

Fig. 4 (case 1).—Higher magnification of figure 3 showing accretion of loose fibrinous meshwork to the endothelial surfaces of the distal portion of the valve and spongy, fragmented center of valve. Hematoxylin and eosin; × 48.

bilicus. The pulse rate was irregular, of Corrigan type and 106 per minute. The blood pressure was 120 systolic and 0 diastolic.

Despite therapy, the patient died on April 21. An electrocardiogram taken the day prior to death showed sinus tachycardia and right ventricular strain.

Observations at Autopsy (by Dr. J. W. Denton and Dr. A. F. Heyl).—The body was that of a well developed, well nourished white woman. The skin, conjunctivas and mucous membranes were pale. There were no petechiae. The superficial lymph nodes were not palpable.

Body Cavities: The peritoneal cavity contained about 200 cc. of clear yellow fluid and the pericardial cavity about 80 cc. of a similar fluid. The right and left pleural cavities contained, respectively, 400 and 150 cc. of similar serous fluid. The serous surfaces were all smooth and glistening.

Cardiovascular System: The heart weighed 560 Gm. The atria were extremely distended, especially the right. The right ventricular wall measured up to 0.6 cm. in thickness and the left 1.8 cm. The myocardium was firm and red-brown. The tricuspid and pulmonary valves were not remarkable. The mitral valve was slightly thickened along the contact borders. The aortic orifice admitted the index finger with ease. The anterior and middle cusps were strikingly deformed. The free portions appeared thickened, foreshortened, gray and shredded, allowing portions of the valve to flap back and forth freely in the blood stream. The shredded valve cusps were granular and appeared to be partially calcified in places. Attached to them was a soft yellow and red blood clot. The coronary ostiums and arteries showed nothing of note. The elasticity of the aorta was good.

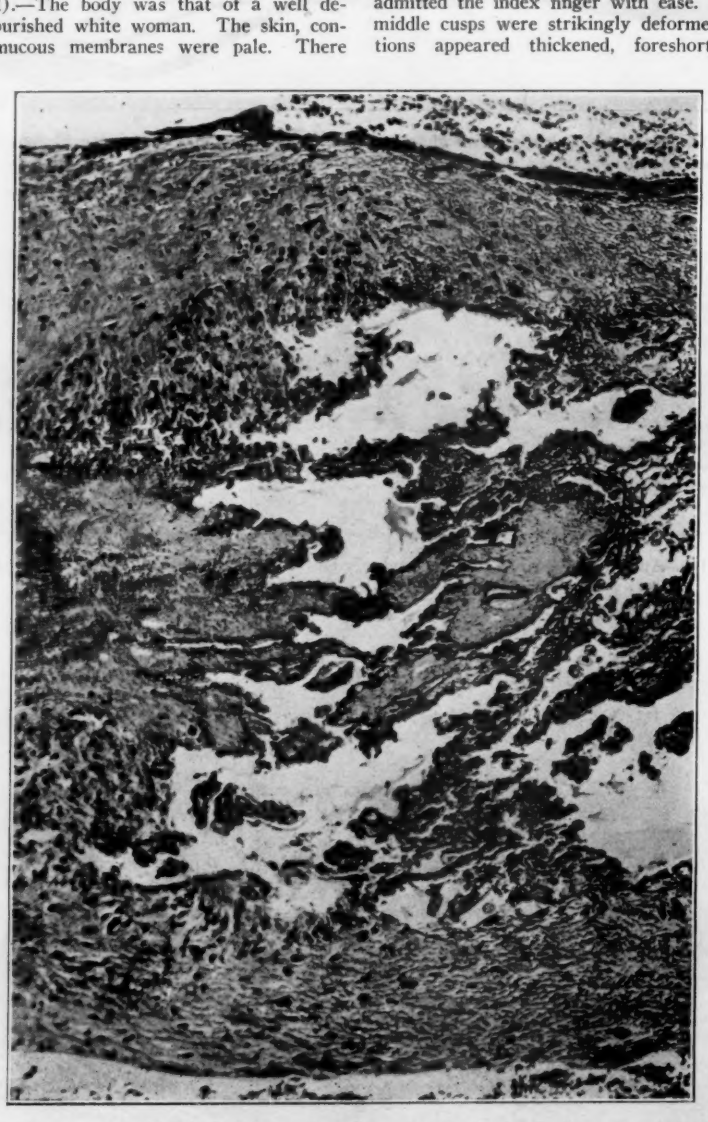

Fig. 5 (case 1).—Higher magnification of figure 4 to show detail of central fragmentation of collagen. Note palisading of fibroblastic cells at periphery. No bacteria are seen. Hematoxylin and eosin; × 120.

Respiratory Tract: Both lungs were pink mottled with dark red externally. Crepitus was diminished, and the lungs cut with moderate resistance. There were, however, no definite areas of consolidation. The cut surfaces were grayish red and moderately wet. In the lower lobe of the left lung were two small, dark red wedge-shaped infarcts. The bronchi contained frothy fluid material. The pulmonary vessels were not remarkable.

Gastrointestinal Tract: The esophagus, stomach and small and large intestines showed nothing of note.

Biliary System: The liver was moderately enlarged, purple-red and smooth. The mottling of passive congestion was readily visible through the capsule and was much more evident on section. The gallbladder and biliary ducts were not remarkable.

capsule was stripped with ease, revealing a congested, red-brown surface, which was slightly granular. In the cut surfaces, cortex and medulla were well demarcated and in the usual proportions. There were no petechiae. The left kidney weighed 210 Gm. and was similar to the right. The renal pelves, ureters and urinary bladder showed nothing of note.

Spleen: The spleen was firm and weighed 400 Gm. The external surface was purple-gray, and the capsule was tense. In the cut surfaces the follicles were large, numerous and distinct. The pulp was deep red and abundant.

Genitalia: The uterus contained two small leiomyomas. The ovaries and fallopian tubes showed nothing of note.

Cranial Cavity: The cranial cavity was not examined.

Fig. 6 (case 1).—Middle aortic cusp with liquefaction necrosis in central portion of cusp and shredding at the periphery. Hematoxylin and eosin; × 11.

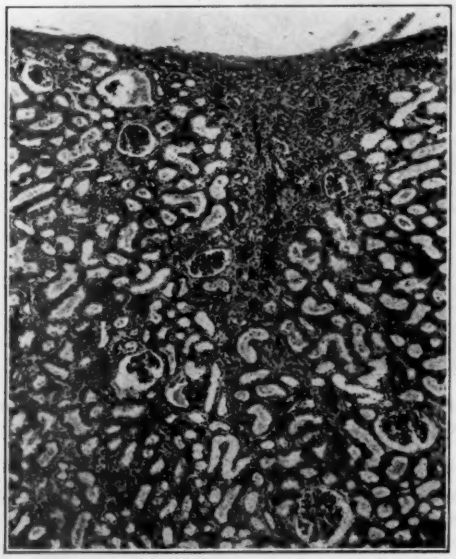

Fig. 7 (case 1).—Kidney, showing small wedge-shaped cortical scar. Hematoxylin and eosin; × 50.

Pancreas: The pancreas was normal in appearance.

Adrenal Glands: The adrenal glands were of usual size and shape and showed nothing of note.

Renal System: The right kidney weighed 190 Gm. The peripelvic adipose tissue was edematous. The

Histologic Examination.—Heart: In a preparation from the left side of the heart, including the mitral valve, the epicardium was found to be of usual thickness. The myocardium was broad and composed of slender fibers which stained well. Striations were moderately distinct. The interstitial tissue was only slightly increased in amount and density, and in places there were a few small perivascular spindle-shaped areas composed of dense fibrous tissue. At the valve ring (fig. 1) the connective tissue was loose and harbored an increased number of small, well formed blood vessels. The valve itself showed nothing of note and did not appear thickened. Examination of the atrial wall revealed some thickening of the endocardium but otherwise nothing of note. Another preparation of the mitral valve (fig. 2) showed a different picture. It was slightly thickened, and at the distal extremity and along its extent there were small subendothelial rounded nodular areas composed of loose fibrous tissue which was arranged in whorls. Section of the anterior cusp of the aortic valve (figs. 3, 4 and 5) revealed a remarkable picture. The valve was thick and composed of dense hyalinized fibrous tissue in its proximal portion. As the free border was approached, the central portion of the valve appeared fragmented, and there were irregular patches of dense, pink-staining acellular collagenous tissue, which were interspersed with clear spaces. At the periphery was denser cellular fibrous tissue. There was some palisading of deeply staining fibroblastic cells toward the center of the valve. Attached to both endocardial surfaces of the valve was a loose fibrinous meshwork on which red and white blood cells were seen. The free edge of the valve was completely shredded, and the continuity of the endocardium had been lost. Sections stained by the Gram-Weigert method failed to reveal organisms

either within the valve or on the surfaces. Preparations from the middle cusp of the aortic valve (fig. 6) were similar. Here the core of the valve appeared cystlike and contained homogeneous pink-staining material. The free edge was likewise fragmented and disorganized. In one place there was a deposit of blue-staining calcific material.

Lungs: Preparations from the two lungs were similar. The alveolar walls were somewhat thickened, and the air spaces were filled with large numbers of mononuclear cells containing golden brown pigment granules. In places the alveoli contained homogeneous pink-staining material. The blood vessels were engorged.

Liver: Sections of the liver revealed severe central necrosis with pronounced dilatation of the central veins and sinusoids.

Kidneys: Sections taken from the two kidneys were similar. There were small scattered cortical wedge-shaped scars (fig. 7) in which were hyalinized glomeruli, thickened small blood vessels and accumulations of small and large round cells. Otherwise the glomeruli were numerous, well formed and well distributed. The intraglomerular capillaries were distended, and the capsular spaces either were empty or contained small amounts of homogeneous or granular pink-staining material. Small amounts of precipitated protein were also seen within the lumens of the convoluted and collecting tubules. No lesions suggestive of focal embolic glomerulonephritis were seen. On the other hand, there were scattered glomeruli which appeared more cellular than usual. In these, the capillary tufts were inconspicuous, and the endothelial cells were large and hyperchromatic. Some of the tufts also contained numbers of polymorphonuclear leukocytes (fig. 8).

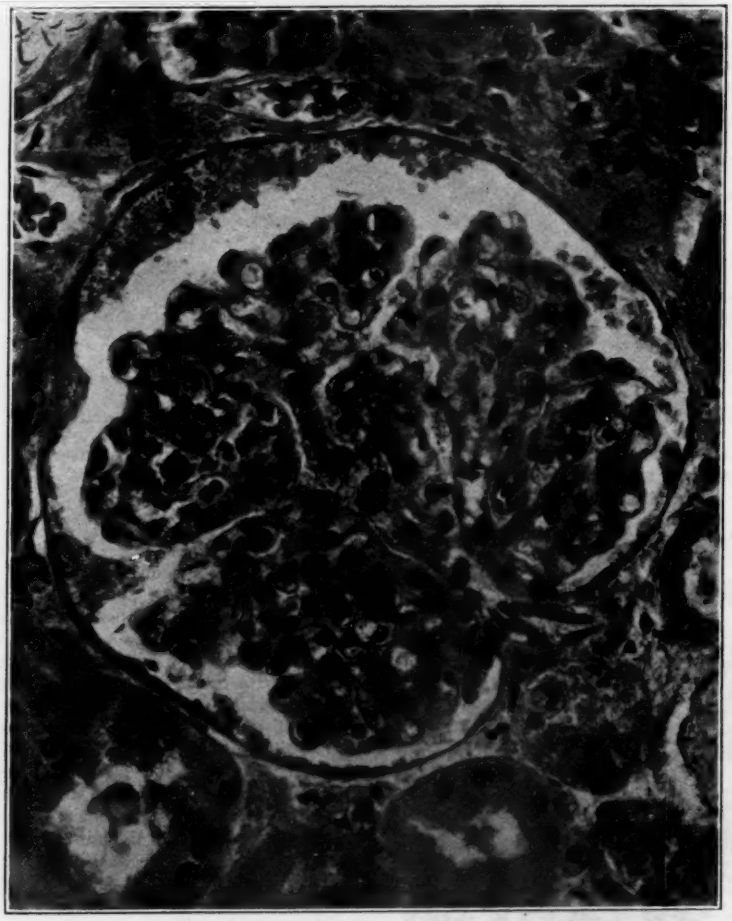

Fig. 8 (case 1).—Glomerulus showing amorphous material within capsular space; note increased cellularity, cohesion of tufts and collections of polymorphonuclear leukocytes. Hematoxylin and eosin; × 450.

Spleen: The follicles were large and increased in number, and there was striking hyperplasia of the germinal centers (fig. 9). The pulp was cellular and hyperplastic, and the sinus walls were difficult to make out.

Postmortem aerobic and anaerobic cultures of the blood and of portions of the aortic valves showed no growth.

The anatomic diagnoses were: fragmentation of the anterior and the middle cusps of the aortic valve, with insufficiency; healed mitral endocarditis; hypertrophy and dilatation of the heart; bilateral hydro-

thorax; ascites; passive congestion of the viscera; stasis infarcts in the left lung; scars in the kidneys, and acute intracapillary glomerulitis.

SUMMARY OF CASE AND COMMENTS

This was a case of subacute bacterial endocarditis due to Str. viridans of at least seven months' duration prior to hospitalization. Therapy with massive doses of sulfonamide drugs failed to sterilize the blood stream. On the patient's admission the right kidney was tremendously enlarged, perhaps owing to infarction; the spleen was palpable, and cultures of the blood showed growth of organisms. She received two courses of combined penicillin-heparin therapy, with totals of 8,940,000 Oxford units of penicillin

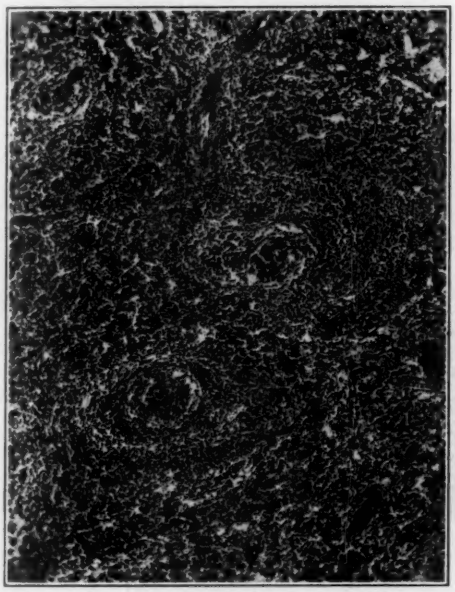

Fig. 9 (case 1).—Spleen, showing follicular hyperplasia. Hematoxylin and eosin; × 53.

and 7,100 mg. of heparin given. She was discharged four months after her admission, bacteria-free and asymptomatic except for moderate cardiac embarrassment due to congestive phenomena. Despite vigorous therapy at home, congestive failure became more apparent, and she died fifteen weeks after treatment, or six weeks after hospitalization.

The demonstration at autopsy of a shredded aortic valve resulting in severe incompetency satisfactorily explained the intractable cardiac failure. That no bacteria could be demonstrated either histologically or by postmortem cultures was evidence confirming the conclusion that the bacterial phase of the disease had been adequately dealt with. Obviously, however, the severe damage already done to the aortic valve was irremediable.

The conditions observed in the kidneys and spleen were of added interest. To begin with, the partial glomerular lesions so conspicuous in many patients with bacterial endocarditis were not present. However, there was evidence of a diffuse glomerulonephritis. This is of particular importance, because, according to Libman and Friedberg,[5] it is much more commonly found in the bacteria-free stage of the disease than in the active phase. Its development after the febrile disease has run its course speaks for a toxic (allergic) origin. The enlarged hyperplastic spleen probably has a similar origin. A remarkable feature of this case was the complete resolution of the massive right renal infarction, necropsy revealing but small cortical scars.

CASE 2.—N. M. was a white woman, aged 29. She was admitted to the Jewish Hospital of Brooklyn on Feb. 16, 1944, transferred from the Good Samaritan Hospital, Lexington, Ky. Her past history was noncontributory except for severe tonsillitis at the age of 2 or 3 years, after which cardiac murmurs were discovered. There was no history of scarlet or rheumatic fever. In November 1943, an infection of the upper respiratory tract developed, for which she was given sulfadiazine. In January 1944, she again suffered an attack of infection of the upper respiratory tract. A low grade persistent fever ensued, which failed to respond to acetylsalicylic acid or to sulfonamide compounds. Chills and fever appeared in February, and she was admitted to the Good Samaritan Hospital for diagnostic study. There it was found that cultures of the blood were positive for Str. viridans. On February 9 she had a sudden cerebral embolization, which rendered her completely aphasic. She was transferred to the Jewish Hospital of Brooklyn seven days later.

At the time of her admission, the patient was well developed and well nourished. She appeared pallid and chronically ill. Her speech was almost completely impaired. The temperature was 101 F., pulse rate 120 per minute, respiratory rate 30 per minute and blood pressure 160 mm. of mercury systolic and 40 diastolic. There were several petechiae on the left hand. The heart was enlarged, and there were double mitral and aortic murmurs. The liver and spleen were both palpable 2 fingerbreadths below their respective costal borders. The reflexes were equal and active, and Babinski signs were not elicited. Culture revealed nine and eleven colonies of Str. viridans per cubic centimeter of blood. The sedimentation rate was 113 mm. per hour (Westergren method), and there was moderate secondary anemia. The urine was normal. The clinical impression was subacute bacterial endocarditis implanted on a rheumatic background, with recent cerebral embolization.

Combined penicillin-heparin therapy by the intravenous route was begun the day after her admission to the hospital. She was given continuous therapy for twenty-three days, receiving 200,000 Oxford units of

5. Libman, E., and Friedberg, C.: Subacute Bacterial Endocarditis, New York, Oxford University Press, 1941.

penicillin per day and from 100 to 200 mg. of heparin. The total dose was 4,600,000 Oxford units of penicillin and 2,900 mg. of heparin. Her response to therapy was most gratifying. Cultures of blood, taken during therapy and afterward, showed no growth. Her speech cleared gradually, and on March 26 it was noted that there was only slight slurring. On April 13, the sedimentation rate was 16 mm. per hour and the temperature curve was flat. The patient was discharged on April 17 for convalescent care at home.

Subsequent Course (by Dr. T. M. Marks, Lexington, Ky.).—On May 17, it was noted that the patient showed remarkable improvement in her appearance. Her heart action was good, but she still had the murmurs from her primary heart disease. The blood pressure was 180 systolic and 70 diastolic. Culture of the blood showed no growth; the sedimentation rate was 8 mm. per hour, and the blood count was normal.

From this time on, her cardiac status gradually deteriorated, and advanced congestive failure developed, which responded only slightly to digitalis and mercurial diuretics. Repeated cultures of the blood were sterile. She died on August 7, approximately four months after having been discharged from the hospital, and twenty-two weeks after treatment.

Observations at Autopsy (by Dr. E. S. Maxwell, Lexington, Ky.).—The body was that of a well developed, moderately emaciated white woman. There was slight edema of the dependent portions. The peritoneum was smooth and glistening, and the cavity contained 800 cc. of clear amber-colored fluid. Each pleural cavity contained about 400 cc. of fluid.

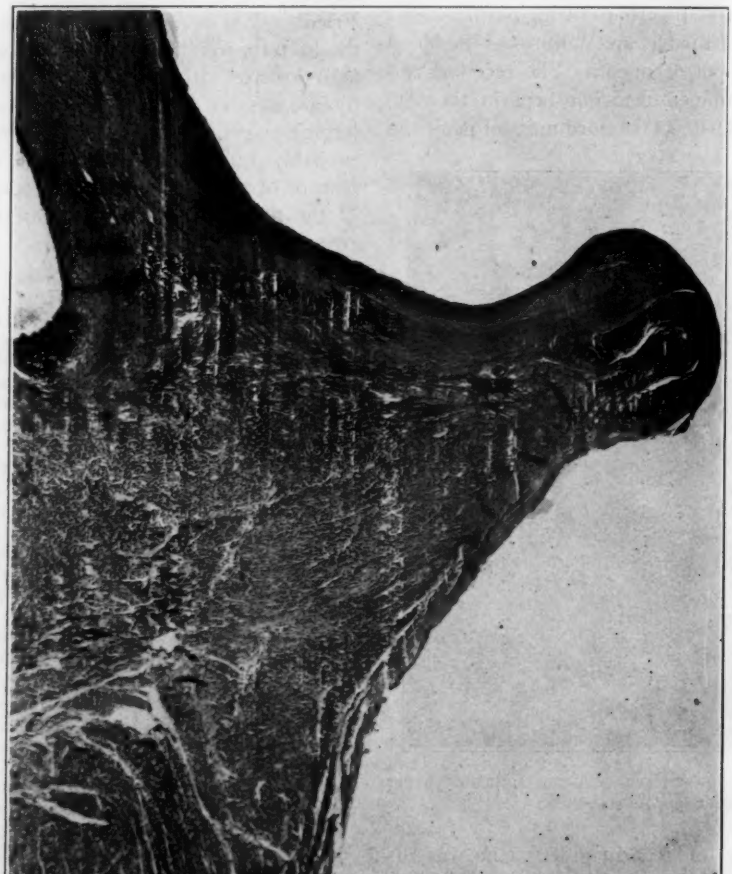

Fig. 10 (case 2).—Aortic cusp; note fibrotic, stumplike character. Hematoxylin and eosin; × 11.

The heart was large, weighing 600 Gm. The mitral leaflets were grossly normal, showing no ulceration, scarring or unusual thickening. The right aortic cusp was almost completely destroyed. The upper margins were short stubs and were brown and finely granular but completely free of vegetation. In the wall of the aorta, immediately above the attached margin of this cusp, there was an opening measuring 3 mm. laterally and 5 mm. perpendicularly. This opened into an

aneurysm-like sac measuring approximately 3 mm. in diameter. This sac contained a small amount of fibrin, which was loosely attached. The wall of the aneurysm was of about the same thickness as the aorta and for the most part was smooth. The sac extended to the right, and the base of the right auricular appendage covered the roof of the aneurysm, while the wall of the

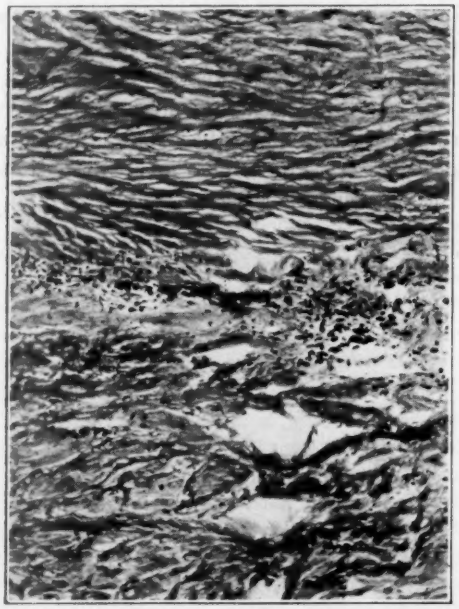

Fig. 11 (case 2).—Aortic valve ring with definite fibrosis and collections of mononuclear cells harboring blood pigment. Hematoxylin and eosin; × 98.

right auricle was adjacent to the aneurysm. The wall of the left ventricle was moderately hypertrophied. The apex was thin. The right side of the heart was grossly normal.

Lungs: The right lung was moderately edematous. On the lateral surface of the upper lobe there was an infarct measuring 2.5 cm. in diameter. The left lung showed moderate edema.

Liver: The liver was approximately normal in size. The cut surfaces show definite nutmeg mottling.

Kidneys: The kidneys were moderately enlarged and were dark purple-red in color. In the cut surfaces, the architecture was normal. The mucosa of the pelvis of each kidney was hemorrhagic. The ureters and urinary bladder were normal.

Spleen: The spleen was moderately enlarged, weighing 250 Gm. Follicles were distinct.

Histologic Examination.—Heart: Sections from the short, thick aortic cusp (fig. 10) revealed dense fibrosis with hyalinization but no inflammatory exudate. The surface was completely endothelialized. In the deeper layers, most prominently at the valve ring, there were collections of mononuclear cells harboring brown pigment granules (fig. 11). Occasional lymphocytes and plasma cells were also seen. Sections of the myocardium showed small scattered areas of fibrosis. Preparations of the wall of the aneurysm revealed it to be identical in thickness and structure with the aorta. No inflammation was seen.

Lungs (fig. 12): The alveolar walls were thickened, and in places there was ingrowth of spindle-shaped fibroblastic cells. The air spaces were filled with "heart failure cells." In some, there were numbers of polymorphonuclear leukocytes enmeshed in fibrin.

Liver: There was extensive passive congestion with central necrosis. The central veins and sinusoids were distended.

Pancreas: The acinar arrangement was orderly. Islands of Langerhans were numerous, well formed and well distributed. The interstitial connective tissue was slightly increased in amount.

Adrenal Glands: The corticomedullary distinction was orderly. The cells of the cortical layer appeared swollen and fragmented in places, and some of the nuclei were pyknotic or absent. The cytoplasm was granular or finely vacuolated. Medullary tissue was loose and abundant.

Kidneys: Sections of the two kidneys were similar. Glomeruli were numerous, well formed and well distributed. Bowman's capsule was slightly thickened in some. The capsular spaces were empty. The glomerular tufts were distended, and the lining cells were occasionally prominent. The epithelium lining the convoluted and collecting tubules was poorly preserved. The blood vessels were not remarkable.

Spleen: The capsule of the spleen was of usual thickness. Follicles were numerous, well formed and well spaced. No germinal centers were seen. The pulp was loose, and the sinusoids were distended. The blood vessels showed nothing of note.

Fig. 12 (case 2).—Lung; noticeable passive congestion with alveoli filled with "heart failure" cells. Note central area of fibrosis. Hematoxylin and eosin; × 100.

The anatomic diagnoses were: congenital aneurysm at the base of the aorta; healed aortic endocarditis; myofibrosis cordis; hypertrophy and dilatation of heart; bilateral hydrothorax; ascites; focal, terminal pneumonia, and passive congestion of the viscera.

SUMMARY OF CASE AND COMMENTS

This was a case of subacute bacterial endocarditis of about one month's duration prior to the patient's hospitalization. An early manifestation of the disease was cerebral embolization, from which she recovered completely with therapy. The patient responded to treatment promptly, one course lasting twenty-three days being sufficient to effect permanent sterilization of the blood stream. The total dosage of penicillin was 4,600,000 Oxford units and of heparin 2,900 mg. Unfortunately, as in case 1, valvular damage was so extensive that compensation was not adequate for the long continuance of life. The stubby aortic cusps found at necropsy allowed free reflux of blood into the left ventricle, and the severe valvular insufficiency resulted in progressive, intractable cardiac failure. The patient succumbed five and one-half months after treatment. An interesting feature was the presence of an aneurysm, probably congenital, at the base of the aorta.

CONCLUSIONS

Two cases of healed subacute bacterial endocarditis were encountered. Death in both instances was due to cardiac failure incident to aortic valvular insufficiency.

STUDIES ON HYPERTENSION

IV. BIOASSAY OF VASOCONSTRICTOR SUBSTANCES IN ULTRAFILTRATES OF CITRATED BLOOD PLASMA FROM PATIENTS WITH NORMAL BLOOD PRESSURES, PATIENTS WITH ESSENTIAL HYPERTENSION AND PATIENTS MADE HYPERTENSIVE BY INTRAVENOUS INJECTIONS OF ANGIOTONIN (HYPERTENSIN)

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Since the results of previous investigations [1] did not support the humoral origin of essential hypertension, it was concluded [1c] that "Final proof awaits conclusive demonstration of the presence or absence of angiotonin in increased amounts in the blood of patients with essential hypertension."

This report deals with a study of whether vasoconstrictor substances are present in increased amounts in the blood of patients with hypertension. The conclusion has been sought through a biologic assay of vasoconstrictor substances in the blood of patients with normal blood pressures, the blood of hypertensive patients and the blood of patients made transiently hypertensive by intravenous injections of angiotonin.

LITERATURE

There are conflicting reports in the literature regarding the presence of pressor compounds in the blood of hypertensive patients or in the blood of animals made hypertensive by renal ischemia. Danzer, Brody and Miles [2] reported that the blood of patients with hypertension contained a pressor substance not found in the blood of rabbits or in the blood of patients with normal blood pressures. Curtis, Moncrieff and Wright [3] repeated the work of Danzer and his associates but found no pressor substance in the blood of patients with hypertension. Both investigations may be criticized, because blood of human beings was injected into animals of another species, the cat. Bohn [4] claimed that he had demonstrated a pressor substance in an alcoholic, protein-free extract of citrated blood from pale hypertensive patients (nephritic) but that similar extracts of blood from both normal persons and plethoric hypertensive patients (with essential hypertension) produced a fall in blood pressure. He, too, injected extracts of human blood into animals of a heterologous species. De Wesselow and Griffiths [5] were unable to confirm Bohn's results. They could adduce no evidence of a pressor substance in the blood of patients with essential hypertension. Ultrafiltrates from plasma showed no effect, with the exception of a slight pressor effect produced by an ultrafiltrate of blood from a patient with chronic glomerulonephritis with slight renal failure. Aitken and Wilson [6] also were unable to confirm the claim of Bohn that blood of pale

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The Eli Lilly Company Laboratory of Clinical Research, I. H. Page, Director, furnished angiotonin for this study.

1. (*a*) Gregory, R.; Lindley, E. L., and Levine, H.: Studies on Hypertension: I. The Effect on the Renal Function of Decreasing the Blood Pressure of Patients with Hypertension, Tex. Rep. Biol. & Med. **1**:59-76, 1943; (*b*) II. The Effect of Spinal Anesthesia on the Blood Pressure of Hypertensive Patients: Its Possible Bearing on the Pathogenesis of Essential Hypertension, ibid. **1**:167-206, 1943. (*c*) Gregory, R.; Levine, H., and Lindley, E. L.: Studies on Hypertension: III. The Site of Action of Angiotonin; the Pressor Effects of Angiotonin on Patients Having Essential Hypertension, and Possible Relationships to the Pathogenesis of Essential Hypertension, ibid. **2**:121-134, 1944.

2. Danzer, C. S.; Brody, J. G., and Miles, A. L.: On the Existence of a Pressor Substance in the Blood of Clinical Cases of Hypertension, Proc. Soc. Exper. Biol. & Med. **23**:454-457, 1926.

3. Curtis, F. R.; Moncrieff, A. A., and Wright, S.: The Supposed Presence of a Pressor Substance in the Blood of Patients with High Blood Pressure, J. Path. & Bact. **30**:55-59, 1927.

4. Bohn, H.: Untersuchungen zum Mechanismus des blassen Hochdrucks: I. Gefässverengernde Stoffe im Blute beim blassen Hochdruck, Ztschr. f. klin. Med. **119**: 100-139, 1932.

5. de Wesselow, O. L. V. S., and Griffiths, W. J.: On the Question of Pressor Bodies in the Blood of Hypertensive Subjects, Brit. J. Exper. Path. **15**:45-52, 1934.

6. Aitken, R. S., and Wilson, C.: An Attempt to Demonstrate a Pressor Substance in the Blood in Malignant Hypertension, Quart. J. Med. **28**:179-190, 1935.

hypertensive patients contained a pressor substance. Page[7] reported that ascitic fluids, as well as blood and cerebrospinal fluids of normal persons, contained a pressor substance. He could not demonstrate any increase of this pressor substance in the blood or cerebrospinal fluid of patients having hypertension. Interestingly, he observed that the medulla and spinal cord were essentially instrumental in the production of this pressor effect. Fasciolo, Houssay and Taquini[8] stated that blood from the renal vein of an ischemic kidney of a dog contained more pressor substance than blood which had passed through a normal kidney of a dog. They used a perfusion technic on the South American toad. Using frogs, bullfrogs and toads from the southern part of the United States of America, Mason and Rozzell[9] were unable to confirm the results of Houssay and co-workers. The use of such widely different species as the dog and the frog is again open to criticism. Page,[10] using a perfused rabbit's ear, concluded that "since vasoconstriction occurs under the same experimental conditions with plasma from both hypertensive patients and dogs, this is considered cogent evidence in favor of the view that the chemical mediator of both is similar and is possibly angiotonin."

While the methods used are perhaps open to question on the grounds mentioned, the results of injecting blood, plasma or extracts of blood and of plasma from hypertensive patients into other animals have failed to demonstrate conclusively the presence of an excessive amount of pressor substance in the blood of hypertensive patients.

METHOD OF STUDY

At the beginning of these studies, citrated plasma from normal persons and from hypertensive patients was injected into small dogs (3 to 4 Kg.) arranged for the kymographic recording of the blood pressure. This procedure was immediately abandoned because of the extreme depressor effects on dogs of plasma from both normal and hypertensive patients.

Ultrafiltrates of citrated plasma were next injected into small dogs. In general, only a suggestive or doubtful increase of blood pressure was produced by the ultrafiltrate from blood to which a known pressor amount of angiotonin had been added, compared to an ultrafiltrate of the same blood without the added angiotonin. The dogs were then abandoned as being unsuitable experimental animals for this purpose.

In all the subsequent experiments we have used Wisconsin winter frogs. They were kept in an ice box at about 4 C. and allowed to be at room temperature from two to four hours before they were to be used. Bioassays were made on them after complete pithing was performed. Perfusion of the entire frog was done at a constant perfusing pressure of 15 cm. with a cannula placed in the truncus arteriosus. The sinus venosus was slit to permit egress of the perfusing fluid, and the webs of the hindfeet were slit to retard the development of edema. The animal thus prepared was perfused on an inclined board, and the number of drops in each two minute interval was recorded with an automatic drop recorder. The volume of perfused fluid collected in a two minute interval was also measured. In general, five intervals of two minutes each were employed for both control and experimental periods, although the perfusion was often extended beyond five two minute intervals.

In nearly all instances the frog was perfused with calcium-free frog Ringer solution[11] to wash out the vascular system as well as for a control against which the experimental solutions were tested. The calcium-free frog Ringer solution contained 0.4 per cent sodium citrate, the same amount as that contained in the blood plasmas from which our ultrafiltrates were made. In some instances, indicated in the proper table, dibasic sodium phosphate (Na_2HPO_4) was added to bring the p_H to about 8.3.

Care was observed to see to it that temperatures of all solutions perfused through any one frog were the same. Perfusions were done at room temperature and ranged from 25 C. to 30 C.

The effect of changes in hydrogen ion concentration on the perfusion rate was studied, and it was found that there was no effect within the limits of 7.3 to 8.4, the range over which our studies were made.

An attempt was made to perfuse these frogs with human plasma according to the method employed by Houssay and Taquini[12] with dog's plasma. We found that even when the calcium-free citrated frog Ringer solution was employed for a 1:8 dilution of the plasma, the vasoconstrictor action was too intense to permit us to use this method. We therefore decided that such dilutions interfered with usefulness and decided to use ultrafiltrates solely.

In all the experiments subsequently reported in this paper we have used the following ultrafiltrates to bioassay the vasoconstrictor substances by the method described:

1. Ultrafiltrates of citrated plasma from 5 persons with normal blood pressures.
2. Ultrafiltrates of citrated plasma from 9 patients with hypertension.
3. Ultrafiltrates of citrated plasma from 8 patients whose blood pressures had been raised by intravenous injections of angiotonin.

7. Page, I. H.: Pressor Substances from the Body Fluids of Man in Health and Disease, J. Exper. Med. **61**:67-96, 1935.

8. Fasciolo, J. C.; Houssay, B. A., and Taquini, A. C.: The Blood-Pressure, Raising Secretion of the Ischaemic Kidney, J. Physiol. **94**:281-293, 1938.

9. Mason, M. F., and Rozzell, J. D.: Attempts to Demonstrate Vasopressor Properties in the Serum of Hypertensive Dogs, Proc. Soc. Exper. Biol. & Med. **42**:142-144, 1939.

10. Page, I. H.: The Vasoconstrictor Action of Plasma from Hypertensive Patients and Dogs, J. Exper. Med. **72**:301-310, 1940.

11. The composition of frog Ringer solution is as follows:

	Regular %	Alkaline %
Sodium chloride	0.65	0.45
Potassium chloride	0.014	0.014
Calcium chloride	0.012	0.012
Sodium bicarbonate	0.020	0.20

This solution was modified for the purposes of these experiments as described in the text.

12. Houssay, B. A., and Taquini, A. C.: Accion vasoconstrictora de la sangre venosa del rinon Isquemiado, Rev. Soc. argent. de biol. **14**:5-14, 1938.

Blood was obtained rapidly (in one to two minutes) in all cases by puncture of the femoral artery. It was drawn into a sterile evacuated bottle containing 10 cc. of 4 per cent solution of sodium citrate per hundred cubic centimeters of withdrawn blood. In general, 200 cc. of blood was withdrawn from each patient into each of two different bottles at an interval of five to ten minutes. The two bleedings were done in order to maintain similar conditions for all subjects, since when we injected angiotonin intravenously we first withdrew 200 cc. as a control and later withdrew an additional 200 cc. of blood when the systolic blood pressure was at its peak of about 180 to 200 mm. of mercury. Patients with normal and elevated pressures were bled similarly in two different 200 cc. amounts in order to have the procedures identical in all groups.

Blood was taken from the femoral artery for two reasons: primarily, because it was believed that the concentration of angiotonin might be higher in arterial blood than it would be in venous blood after the blood had circulated through the peripheral tissues; and, secondly, so that a 200 cc. sample could be obtained quickly during the peak of the blood pressure elevations resulting from the intravenous injection of angiotonin.

Immediately after the blood was withdrawn, it was placed in cracked ice with a minimum of agitation to prevent hemolysis. As rapidly as possible, the blood was centrifuged in sterile 50 cc. tubes. The citrated plasma was transferred to the ultrafiltration apparatus with precautions for asepsis. No plasma prepared in this fashion showed hemolysis. We have employed an ultrafiltration apparatus previously described by one of us.[13]

Ultrafiltration was carried out in bags made from cellophane "osmosis membrane" and of such length and diameter as to take 100 to 125 cc. of citrated plasma. All ultrafiltrations were done in a mechanical refrigerator with the temperature at about 4 C.

RESULTS

Control Studies.—It was established by trial that 5 to 10 cc. of the available angiotonin solution would raise the systolic blood pressure of an average adult to levels of 180 to 200 mm. of mercury. It was felt that this amount represented a dilution of 1:500 to 1:1,000 of the injected solution of angiotonin. Accordingly, it was considered necessary to establish whether our method of bioassay would detect dilutions of this order. The results in table 1 indicate in general the ranges of effective strengths. Dilutions of 1:10,000 angiotonin in calcium-free citrated frog Ringer solution were not effective; of 1:1,000 were usually effective, and of 1:500 were always strikingly effective. It may be noted further that the vasoconstrictor effects of angiotonin were always manifested by the second two minute perfusion interval and that there was always a recovery to or toward the preultrafiltrate levels when ultrafiltrates were followed by calcium-free citrated frog Ringer solution.

Analysis of the data in table 1 will show that frequent assays of a known dilution of the angiotonin were done over the period during which our experiments were in progress. They were done to make sure our angiotonin did not deteriorate in strength. We had no reason to believe it would, because our stock was kept at 4 C. and all withdrawals were made with strict precautions for asepsis. They were done to make sure also that the various lots of frogs remained responsive to angiotonin.

TABLE 1.—*Effects on the Perfusion Rate of 1:1,000 and 1:500 Angiotonin in Calcium-Free Citrated Frog Ringer Solution*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals

Frog	No. 1	No. 2	No. 3
Date	4/15/44	5/17/44	5/19/44
Control with frog Ringer solution	7.2	10.5	6.3
	7.0	11.0	6.4
	7.2	11.3	6.4
	7.2	11.7	6.4
	7.0	12.4	6.5
		12.7	
1:1,000 angiotonin	3.2	11.0	6.2
	3.0	8.7	5.2
	2.3	7.8	5.6
	2.2	...	4.8
	2.0	...	5.0
Return to frog Ringer solution	3.0	8.4	5.8
	3.0	10.3	5.2 †
	3.0	10.2	5.5
	3.2	10.0	5.7
	3.4	10.8	5.7
	...	11.0	5.8

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals

Frog	No. 4	No. 5	No. 6	No. 7	No. 8
Date	5/19/44	5/20/44	5/19/44	6/15/44	6/15/44
Control with frog Ringer solution	9.3	11.9	12.2	9.4	8.0
	9.3	11.9	12.5	8.8	7.4
	9.3	6.4*	11.9	8.7	7.8
	9.3	9.8	11.1	9.2	7.8
	9.5	8.4	11.1	9.4	7.8
			10.6	9.4	7.8
				9.4	
1:500 angiotonin	8.5	6.4	11.2	9.2	7.4
	5.5	4.9	9.4	7.3	6.2
	4.9	4.3	8.0	6.0	5.3
	4.7	4.3	7.0	5.8	4.9
	4.7	4.3	6.4	5.7	4.7
Return to frog Ringer solution	5.3	4.5	6.2	5.2	4.7
	6.3	5.6	7.8	6.1	5.2
	6.7	5.5	9.2	6.9	5.6
	6.8	5.4	9.8	7.2	5.7
	7.0	5.8	9.5	7.4	5.7
				7.5	5.8
					5.6

* Ran out of perfusion fluid.
† Pressure down.

Detailed consideration of the figures will show that whereas there is evidence that 1:1,000 dilution of our angiotonin solution frequently produced vasoconstriction, 1:500 dilutions never failed to produce vasoconstriction, as shown by a decrease in the volume of perfusate during the ten or more minutes of the test. It may also be seen that the volume of perfusate in a two minute period invariably increased after the return to calcium-free citrated frog Ringer solution from the known angiotonin solution. It usually did not return to the volume of perfusate

13. Gregory, R., and Andersch, M.: The Filterable Calcium of Blood Serum, Am. J. M. Sc. **191**:263-271, 1936.

obtained before the angiotonin perfusion was started. But there was always a definite increase, a result contrary to that which would be obtained if the decrease during the period of perfusion with angiotonin was a manifestation of the frequently observed tendency of the perfusate volume to decrease gradually with time when calcium-free citrated frog Ringer solution alone is employed.

From the data presented in table 1 it is concluded that (1) our frogs were sensitive to the vasoconstrictor action of the available angiotonin in dilutions of as much as 1:1,000 and always in dilutions of 1:500 of the original stock of calcium-free citrated frog Ringer solution and (2) that our solutions of angiotonin remained potent during the period of our experiments.

After it had been established that the frogs were sensitive to dilutions of 1:1,000 to 1:500 of angiotonin and inasmuch as our earlier experiments made us decide to use ultrafiltrates of citrated plasma, we next found it necessary to prove that angiotonin was ultrafiltrable. Consequently a known amount of angiotonin was added to calcium-free citrated frog Ringer solution and to human blood. These solutions were then treated as indicated in our discussion of methods. Four hundred cubic centimeters of blood was drawn as usual and citrated to usual strength. This was divided into two portions. The first 200 cc. was chilled, centrifuged and ultrafiltered as described. To the second 200 cc. we added 2 cc. of angiotonin and prepared an ultrafiltrate. Ultrafiltrates from these two different 200 cc. portions were perfused through frogs. The results when angiotonin was added to calcium-free citrated frog Ringer solution are shown in table 2 A. The results when angiotonin was added to blood are shown in table 2 B.

Analysis of data in table 2 A, according to our interpretation, shows that angiotonin is ultrafiltrable in calcium-free citrated frog Ringer solution. And while we do not believe we have done experiments numerous enough to be statistically significant, the data suggest that angiotonin does not filter quite as rapidly as water. This suggestion is based on the finding that

TABLE 2 A.—*The Vasoconstrictor Effects of (a) 1:500 Solution of Angiotonin in Calcium-Free Citrated Frog Ringer Solution, (b) Ultrafiltrate of a 1:500 Solution of Angiotonin in the Same Frog Ringer Solution and (c) Solution Remaining in the Sac After Twelve Hours of Ultrafiltration of a 1:500 Angiotonin Solution in the Same Frog Ringer Solution*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals

Frog	No. 1	No. 2
Date	8/1/44	8/1/44
Alkaline frog Ringer solution with 0.4% sodium citrate + 2 cc. fifth-molar solution of dibasic sodium phosphate (Na_2HPO_4) per liter at p_H 8.5; T. 38 C.	5.2 6.4 6.0 7.0 7.3 7.4	10.5 10.4 10.4 10.1 10.0
1:500 angiotonin in frog Ringer solution as above p_H 8.5	7.2 5.8 5:1 5.0	8.4 6.2 5.6
Control frog Ringer solution p_H 8.5	6.4 7.0 7.6 7.6 7.7	5.1 6.8 7.8 7.8 7.8 7.7

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals

Frog.....	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
Date, 1944...	8/2	8/2	8/2	8/2	8/4	8/4	8/4	8/4
Same frog Ringer solution as above	13.2	10.0	....	...	7.2	....	10.3	...
	12.6	8.7	....	...	7.2	....	9.8*	...
	12.6	7.6	....	...	7.0	....	8.1	...
	12.2	7.4	....	...	7.0	....	8.2	...
	11.8	7.4	....	...	6.8	....	8.0	...
	11.3	7.0	....	...	6.8	....	...	...
Ultrafiltrate of 1:500 angiotonin in frog Ringer solution	11.2	6.6	....	...	5.8	....	8.0	...
	11.0	5.4	....	...	5.0	....	7.0	...
	9.8	5.6	....	...	4.0	....	6.4	...
	9.4	5.8	....	...	...	....	...	...
Control frog Ringer solution	9.4	7.0	10.8	6.3	4.6	15.6	6.6	6.2
	9.6	7.3	10.6	5.8	5.0	14.0	7.1	6.2
	9.2	7.3	10.1	5.5	5.0	12.5	7.4	7.8
	8.2	7.4	9.2	6.3	5.1	12.8	7.0	8.0
	7.8	...	8.6	6.1	5.2	13.4	7.3	7.8
	7.3	...	...	6.4	5.2	13.4	...	...
	7.4	...	...	...	...	....	...	...
	7.2	...	...	...	...	....	...	...
Sac residue from ultrafiltrate of 1:500 angiotonin above	7.0	7.3	6.0	6.2	...	12.5	...	8.0
	6.0	6.2	4.0	4.8	...	9.8	...	7.6
	4.6	4.8	3.4	4.0	...	8.8	...	7.2
	4.0	...	...	3.6	...	...	...	7.2
Control frog Ringer solution	4.0	4.8	3.6	3.8	...	8.8	...	6.6
	3.8	5.2	3.6	4.3	...	9.6	...	6.8
	4.0	5.4	3.6	4.4	...	10.2	...	7.0
	3.8	5.6	3.6	4.4	...	10.1	...	7.2
	3.7	5.2	3.3	4.3	...	9.8	...	...
	...	5.4	...	4.3	...	...	...	...

* Retied cannula.

TABLE 2 B.—*Vasoconstrictive Effects of an Ultrafiltrate of Blood Plasma and an Ultrafiltrate of Plasma of the Same Blood to Which Angiotonin Had Been Added in Proportion of One Cubic Centimeter to One Hundred Cubic Centimeters Blood*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals

	Frog No. 1		Frog No. 2
Control with calcium-free citrated frog Ringer solution	5.3		15.8
	5.4		14.2
	5.5		15.4
	5.7		15.2
	5.9		15.0
	6.1		
	6.3		
Ultrafiltrate of plasma from plain blood	6.1	Ultrafiltrate from blood to which 2 cc. angiotonin/200 cc. blood had been added	12.0
	6.2		6.4
	6.0		5.2
	5.8		5.0
	5.3		4.6
	5.0		4.8
Return to frog Ringer solution	4.9		6.4
	5.0		10.4
	4.7		12.4
	5.2		13.2
	5.0		13.1
	5.0		14.3
	5.0		
	5.0		

the solution remaining in the sac is slightly more vasoconstrictive than either the original solution or an ultrafiltrate.

We have concluded from the data given in table 2 A and table 2 B that angiotonin is ultrafiltrable by our method, can be recovered in the ultrafiltrate from blood to which it has been added and can be detected in the ultrafiltrates from blood plasma by our method of bioassay.

The relative usefulness of our method of ultrafiltration of citrated blood plasma compared to the use of 1:8 dilutions of plasma as employed by Houssay and Taquini to demonstrate vasoconstrictor substances in the blood from the renal veins of dogs prepared according to Goldblatt's method can be seen in table 3.

These results show that the diluted blood plasma to which angiotonin had been added is no more effective than the same diluted plasma without angiotonin. Comparison of these results with those in table 2 B will undoubtedly allow one to conclude that the vasoconstrictor substances in citrated human plasma are in such strength that it is almost useless to try to remove them by dilution. It is obvious that an ultrafiltrate from whole plasma contains much less vasoconstrictor substance than a 1:8 dilution of plasma. An ultrafiltrate has the further advantage in studies such as ours that the substance in the bioassay of which we are interested is not significantly diluted, but is presumably present in the ultrafiltrate in about the same strength as it is in the whole plasma.

TABLE 3.—*Effect of One to Eight Dilutions of Citrated Blood Plasma and Citrated Blood Plasma Containing Angiotonin on the Perfusion Rate*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals	Frog No.1
Control with calcium-free citrated frog Ringer solution	8.0
	8.4
	8.2
	6.8
	5.3
	7.8
	7.6
1:8 dilution of patient's plasma	7.2
	7.4
	6.8
	5.5
	4.0
	2.6
	Frog No. 2
Control with the same frog Ringer solution	7.6
	8.0
	7.5
	7.6
	7.6
	7.8
1:8 dilution of same patient's plasma from blood to which 1 cc. angiotonin/100 cc. blood had been added	6.3
	6.0
	5.6
	4.2
	2.8
	1.8

EFFECT OF CHANGES IN HYDROGEN ION CONCENTRATION ON PERFUSION RATE

Early in our experiments it was decided that it was necessary to take into account the possible effect of changes in hydrogen ion concentration on perfusion rate. It was then found that the calcium-free frog Ringer solution that we made with added 0.4 per cent sodium citrate varied in p_H from 7.3 to 7.6 approximately. Most of our plasma ultrafiltrates were found to range from p_H 8.3 to p_H 8.4. In most instances, the p_H of ultrafiltrates from the two different 200 cc. samples of blood drawn from the same subject were practically identical. Because of this small difference, the effect of slight changes in p_H on perfusion rate was studied. The results are indicated in table 4. They are representative of many additional experiments which showed that changes in p_H from 7.3 to 8.3 had no effect or might, as we have just stated, slightly increase the rate of perfusion.

TABLE 4.—*Effect of Slight Changes in the p_H on the Perfusion Rate*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals	
Fresh Calcium-Free Citrated Frog Ringer Solution, p_H 7.35	Frog Ringer Solution That Had Been Made Up Several Days, p_H 8.3
5.0	7.2
4.8	7.0
6.4	7.2
6.0	7.2
6.0	7.0

(Same frog perfused with solutions of different p_H)

In view of these minor variations and of the additional fact that almost all of our ultrafiltrates from normal, angiotonin-treated and hypertensive patients had a p_H of about 8.3, it was thought that this similarity in concentration in itself was sufficient control. However, most of our work was done with calcium-free frog Ringer solution which contained 0.4 per cent sodium citrate (the same amount as that in citrated plasma) and 2 cc. of fifth-molar solution of dibasic sodium phosphate (Na_2HPO_4) per liter and having a p_H of about 8.3.

To summarize, we have concluded that any change in hydrogen ion concentration from calcium-free citrated frog Ringer solution to ultrafiltrates did not account for any of the observed changes. But to avoid any possible criticism most of our perfusion work was done using as perfusion control a phosphate-buffered calcium-free frog Ringer solution containing 0.4 per cent sodium citrate and having p_H 8.3 almost uniformly.

EFFECT OF SODIUM CITRATE IN CALCIUM-FREE FROG RINGER SOLUTION ON PERFUSION RATE

Because the citrated plasma employed contained 0.4 per cent sodium citrate, it was necessary to add that much sodium citrate to our calcium-free frog Ringer solution. In view of the identity of strength in both solution and plasma, it might not have been necessary to control this. Because of the effect of sodium citrate on ionized calcium, however, we did test the effect of a calcium-free frog Ringer solution as well as the addition of sodium citrate to a calcium-free frog Ringer solution on the perfusion rate. Table 5 contains some of the experimental data bearing on these points.

Examination of the data in table 5 shows that the results obtained by perfusing frogs with calcium-free frog Ringer solution containing 0.4 per cent sodium citrate were not significantly different from those obtained with regular frog Ringer solution. The volume of perfusate was within the same range; the volume in each two minute interval tended to stabilize for about the same number of minutes and to show a tendency to gradual decline in volume with the passage of time.

TABLE 5.—*The Effects of Four Tenths Per Cent Sodium Citrate on the Perfusion Rate*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals

Frog.....	No. 1	No. 2	No. 3	No. 4	No. 5
Regular frog Ringer solution *p*H 7.35	7.0	4.7	5.9	8.0	11.7
	7.4	5.4	5.2	8.4	15.8
	7.8	4.6	5.2	8.2	16.4
	7.6	6.0	4.9	8.0	15.8
	Spilled	5.5	4.0	7.8	14.2
	8.5	...	...	7.6	15.4
	8.6	...	...	...	15.2
	9.8	...	...	...	15.0
	Spilled				
	10.6				
	12.7				
	12.2				
	12.7				
	12.5				
	11.2				
	11.0				

Frog.....	No. 6	No. 7	No. 8	No. 9
Calcium-free frog Ringer solution + 0.4% sodium citrate	6.4	6.2	11.6	7.6
	6.0	7.7	11.9	8.0
	5.8	7.9	12.2	7.5
	6.0	7.8	12.5	7.6
	5.8	7.8	11.9	7.6
	5.9	8.1	12.0	7.8
	5.8	...	11.6	
	5.9			
	5.7			
	5.8			
	5.6			
	5.4			
	5.2			
	5.4			
	5.5			
	5.4			
	5.3			
	5.3			
	5.2			
	5.3			
	4.7			
	4.9			
	4.8			
	4.7			

Finally, it was considered necessary to study as a control the perfusion behavior when frogs were perfused for forty-five to sixty minutes with calcium-free citrated frog Ringer solution. Table 6 shows the data obtained in this study. It can be seen from this table that the volume of the two minute perfusates is constant at least for the first twenty minutes. In some frogs it is constant for periods considerably longer than twenty minutes. In other frogs there is a very gradual decline after the first ten periods. It may be concluded that the perfusion method is constant enough in the first twenty minutes or more to make apparent the effects of any vasoconstrictor substances present in plasma ultrafiltrates, which were always perfused through the frog within the time limits in which the data showed the perfusate volumes to be reasonably uniform.

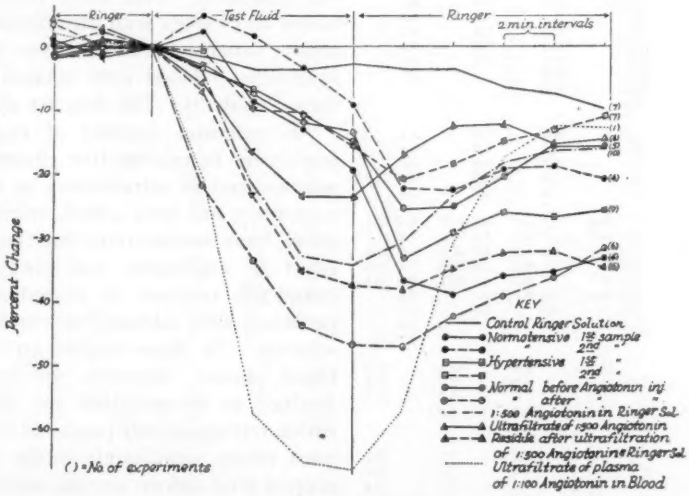
TABLE 6.—*The Effect of Time on the Perfusion Rate with Calcium-Free Frog Ringer Solution Containing Four Tenths Per Cent Sodium Citrate and Two Cubic Centimeters Fifth-Molar Dibasic Sodium Phosphate (Na_2HPO_4) Per Liter. pH Ranging From 7.7 to 8.3*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals

Frog	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Date	6/13/44	6/6/44	6/13/44	5/26/44	5/26/44	5/11/44
	9.4	10.4	8.4	6.9	6.6	16.4
	10.0	9.6	8.6	7.8	7.0	16.5
	9.8	10.1	8.6	8.6	7.4	16.4
	10.1	10.8	9.1	8.4	7.4	17.5
	10.0	9.6	9.0	8.4	6.8	18.4
	10.1	9.6	8.8	7.9	6.9	16.8
	10.4	10.1	8.6	7.5	6.7	15.6
	10.4	10.1	8.2	7.8	6.2	17.4
	11.0	10.0	8.2	8.0	6.5	17.9
	11.0	9.9	7.8	7.9	6.4	18.2
	11.2	9.6	7.6	7.9	6.8	17.8
	11.2	9.2	6.6	7.9	6.9	17.4
	11.1	8.9	6.3	7.8	6.9	17.4
	10.7	8.4	6.2	7.8	6.8	17.0
	10.7	8.6	Lost	7.8	6.2	17.3
	10.8	8.5	5.9	7.3	6.6	15.7
	10.9	8.6	5.4	6.5	6.8	14.9
	10.8	8.1	5.5	5.8	6.6	15.2
	10.7	8.3	5.4	5.6	6.8	13.9
	10.2	7.7	5.2	5.1	6.7	13.2
	10.3	7.7	5.2	5.5	6.8	11.5
	10.2	7.6	5.0	4.9	6.8	10.2
	9.9	...	Considerable	4.8	6.7	10.0
	9.9	...	edema	4.4	6.6	9.4
	9.3	...	...	4.0	6.2	8.0
					6.0	8.0
					5.8	8.2
					5.4	8.2
					5.3	8.3
					5.1	7.8
					5.0	6.4
					5.0	5.4
					5.1	4.8
					4.8	4.6
					4.8	4.6
					4.4	4.6
					4.3	
					4.0	

From our control studies we have concluded (1) that perfusion of frogs by the method described results in uniform rates of perfusion, (2) that changes in hydrogen ion concentration from 7.3 to 8.3, the range of difference between some of our control frog Ringer solutions and

our plasma ultrafiltrates, apparently has no effect on the perfusion rate, (3) that calcium-free frog Ringer solution containing 0.4 per cent sodium citrate apparently has no inherently vasoconstrictor effect, (4) that whole or 1:8 dilutions of human plasma are entirely unsuitable for perfusion through the frog for our purposes because of the considerable amount of vasoconstrictor substance in citrated human plasma, (5) that frogs prepared for bioassay as we have described are often sensitive to dilutions of 1:1,000 and always sensitive to dilutions of 1:500 of the available angiotonin solution, (6) that the amount of angiotonin present in the blood when we have injected it and the amount found necessary to raise the patients' blood pressures to 180 to 200 mm. of mercury systolic was about 1:500 dilution in whole blood or 1:250 in plasma and (7) that angiotonin added to human blood is ultrafiltrable and may be detected in plasma ultrafiltrates by the bioassay method used.



Percentage change in volume of perfusion (in relation to flow immediately before test fluid).

EXPERIMENTAL STUDIES

As a result of the aforementioned control studies, we have proceeded with experimental observations of bioassay of vasoconstrictor substances in ultrafiltrates of citrated plasmas from patients of three types: those with (1) normal blood pressure, (2) hypertension and (3) hypertension produced by intravenous injections of angiotonin.

From all patients the blood was drawn in two different 200 cc. amounts, one immediately after the other. Ultrafiltrates of the plasma from both 200 cc. portions were made and perfused in order to have a control of the effect of drawing the second 200 cc. portion. We were primarily concerned with this second 200 cc. portion in our patients in whom angiotonin had been injected, because the first 200 cc. was used as a control. Drawing so much blood might in itself theoretically cause the body to produce vasoconstrictor substances; accordingly we used this method of withdrawing two different 200 cc. portions of blood from all of our three different types of patients. The work of Sapirstein, Ogden and Southard [14] and that of Collins and Hamilton [15] should be cited in this connection. These workers have demonstrated that large hemorrhages in experimental animals result in an increase of angiotonin. On the other hand, Page [16] reported that nephrectomy did not prevent the appearance of vasoconstrictor substances in the plasma of dogs that had been bled. On the basis of these results, Page has suggested that at least two substances are involved in hemorrhagic shock—a pressor substance from the kidney and a vasoconstrictor substance from elsewhere in the body.

I. Effect of Ultrafiltrates of Plasma from Patients with Normal Blood Pressures.—Ultrafiltrates of plasma from patients with normal

14. Sapirstein, L. A.; Ogden, E., and Southard, F. D., Jr.: Renin-Like Substance in Blood After Hemorrhage, Proc. Soc. Exper. Biol. & Med. **48**:505-508, 1941.

15. Collins, D. A., and Hamilton, A. S.: Changes in the Renin-Angiotonin System in Hemorrhagic Shock, Am. J. Physiol. **140**:499-512, 1944.

16. Page, I. H.: The Occurrence of a Vasoconstrictor Substance in Blood During Shock Induced by Trauma, Hemorrhage and Burns, Am. J. Physiol. **139**:386-398, 1943.

pressures were studied according to the method described. The results are shown in table 7 and in the chart. Studies of blood from 7 other normal patients were also made. These will be referred to again as the individual patient controls in connection with the angiotonin-treated group.

Analysis of the data on the vasoconstrictor effect of ultrafiltrates of citrated plasma from 5 patients with normal blood pressures presented in table 7 shows individual variation. The pres-

TABLE 7.—*Effect of Ultrafiltrates of Citrated Plasma from Patients with Normal Blood Pressures*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals (Each column represents the perfusion of a different frog)					
Patient.....	L. H.	A. R. B.	S. F.	L. H. H.	S. C.
Date.....	5/31/44	6/6/44	6/9/44	6/15/44	6/30/44
Blood pressure..	115/60	110/70	94/62	120/80	110/70
Control perfusion buffered calcium-free citrated frog Ringer solution	10.0 10.0 9.2 9.0 9.0 9.2	9.4 8.9 9.4 10.3 10.7 10.3 10.2 9.9	12.1 12.4 12.0 11.2 11.1 10.9 10.6	7.4 7.4 7.4 7.2 7.5 ...	4.2 4.4 4.2 4.5 4.6 4.7
Perfusion with ultrafiltrate from first 200 cc. sample	10.3 9.0 9.2 9.0	9.8 8.1 6.5* 5.6 4.5	10.5 9.2 8.0 7.0 7.0	7.8 7.2 6.8 5.9 5.2	5.4 4.0 3.9 3.8 3.8
Buffered frog Ringer solution	7.8 8.1 8.4 7.8 8.0	5.3 10.4 4.3 10.7 4.2 9.6 3.9† 10.2 4.3 10.3 4.5 10.2 4.0	6.1 5.4 5.9 7.0 6.4 8.4 6.2 8.2 6.7 8.3 6.4 8.7 6.4	4.2 10.8 4.1 10.6 4.6 10.8 5.0‡ 10.6 4.9 10.1 4.6 4.6	2.6 13.4 2.2 11.3 2.0 10.6 2.2 10.6 2.5 9.4 2.4 2.2 2.0 2.2
Perfusion with ultrafiltrate from second 200 cc. sample	...	11.2 11.1 10.9 10.5	8.8 8.2 7.6 6.4 5.9	11.3 9.9 9.3 8.8	13.0 12.0 11.2
Return to frog Ringer solution	...	9.1 9.5 9.9 9.9 9.7 10.1 9.6	4.4 4.2 4.6 4.7 4.7 4.5 4.6 4.5	8.2 8.6 9.2 9.4 9.1 8.8 9.0	11.3 10.2 9.4 9.3 9.1 9.1 8.8 8.8 9.0 8.6 9.0 8.4

* Severe swelling of abdomen.
† Cannula moved.
‡ Fluid bloody.

ence of slight amounts of vasoconstrictor substances in ultrafiltrates of normal plasma is suggested by the data. In general, however, there is only very slight decrease in the volume of perfusate within the first six minutes of the perfusion. As a matter of fact, in some instances there was a slight increase in the perfusion volume for the first four minutes in the ultrafiltrates from both the first and the second 200 cc. specimen. The slight decrease thereafter is thought to be due to the normal trend of perfusion volumes to decrease slightly with time, even with calcium-free citrated frog Ringer solution plus the slight amounts of vasoconstrictor substances in normal plasma. It should be emphasized that the amount of the decrease and the suddenness with which it occurs after return to frog Ringer solution from plasma ultrafiltrate is not at all characteristic of the effects of angiotonin in calcium-free citrated frog Ringer solution or in plasma ultrafiltrates.

The average effects of the ultrafiltrates from both the first and the second 200 cc. bleeding from 5 patients with normal blood pressures for four different two minute perfusion intervals are shown graphically in the chart. These curves will be commented on later.

II. Effect of Ultrafiltrates of Plasma from Patients with Hypertension.—Nine patients with hypertension were studied according to the methods described. This study represents the perfusion of 14 frogs with ultrafiltrates from hypertensive patients, inasmuch as two different 200 cc. specimens of blood were obtained from most of these 9 patients. The data are shown in table 8.

An extensive number of experiments with angiotonin in calcium-free citrated frog Ringer solution and in ultrafiltrates of blood to which angiotonin had been added, referred to in other tables, have demonstrated that the vasoconstrictor effect of angiotonin was always partially or completely reversed by immediately subsequent perfusion with calcium-free citrated frog Ringer solution. In these studies on ultrafiltrates of blood plasma, therefore, we have believed it justified to demand that any decrease in perfusion rate apparently produced by an ultrafiltrate must return significantly in the direction of the control level before we can attribute a decrease of volume during the ultrafiltrate perfusion to angiotonin.

If the data in table 8 are analyzed, it may be seen that in practically no instance was there any greater decrease in perfusion volume from the ultrafiltrate from either the first or the second 200 cc. sample obtained from the 9 hypertensive patients studied than was produced by ultrafiltrates of plasma from patients with normal blood pressures.

The slight decrease in perfusion volume that did occur is probably due to a well demonstrated tendency of a slight decrease to occur with time in all of these experiments. This decrease is probably due to the absence of protein in the perfusion fluids and the consequent loss of fluids from the vascular channels as well as to the slight amount of vasoconstrictor substances in all plasma. This explanation is supported by the invariable finding of edema of the various

tissues with the passage of perfusion time and by the greater decrease with plasma ultrafiltrates than with calcium-free citrated frog Ringer solution.

Study of the curves in the chart are revealing. It should be understood that these curves represent averages of values obtained from the various groups of patients studied. While there are some variations in the curves for the first two minutes, we believe it highly significant that the curves obtained from plotting the data from the first 200 cc. sample from one group of 4 normal patients and from another group of 6 normal patients (into whom angiotonin was then injected) are practically identical with the curves obtained from plotting the data obtained from perfusion of 14 frogs with plasma ultrafiltrates from patients with hypertension. These curves do not differ essentially from the curve obtained from plotting the ultrafiltrates of the second 200 cc. blood sample from 4 patients with normal blood pressures.

We believe, therefore, that we are justified in concluding that ultrafiltrates of plasma from hypertensive patients do not contain any more vasoconstrictor substances than do ultrafiltrates of plasma from patients with normal blood pressures. The curves representing the effects of plasma ultrafiltrates from normal and from hypertensive patients are different from curves representing the effect of a known angiotonin solution either in calcium-free citrated frog Ringer solution or in an ultrafiltrate from blood to which angiotonin had been added. This permits the conclusion that the vasoconstrictor substance in ultrafiltrates of plasma from normal and from hypertensive patients is probably not angiotonin.

TABLE 8.—*Effect of Ultrafiltrates of Plasmas from Patients with Hypertension*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals														
Patient.....	G. S.		H. P.	A. L.	F. F.		A. W.		N. R.		L. H.		C. H.	M. W.
Blood pressure..	200/100		190/120	285/165	192/96		200/100		184/120		240/150		200/125	250/150
Date.....	5/12/44		5/14/44	5/15/44	5/19/44		5/23/44		5/27/44		7/7/44		5/6/44	3/16/44
Frog.....	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13	No. 14
Perfusion with buffered frog Ringer solution containing sodium citrate, p_H 7.75	5.3	....	15.1	4.6	7.8	....	6.8	....	8.0	...	11.7	....	7.6	4.4
	5.4	....	14.1	5.6	7.8	....	6.7	....	8.2	...	10.3	....	8.2	5.5
	5.5	....	13.8	6.2	7.9	....	6.7	....	8.6	...	10.7	....	7.9	6.4
	5.7	....	14.0	6.5	7.9	....	6.6	....	8.3	...	10.3	....	8.1	3.6
	5.9	....	14.8	6.7	7.8	....	6.5	....	8.3	...	10.6	....	8.2	5.6
	6.1	....	14.2	6.3	...	....	6.4	....	8.6	...	11.0	....	7.8	7.4
	6.3	....	14.6	...	...	....	...	....	8.4	...	11.0	....	...	7.4
	...	....	15.4	...	...	....	...	....	...	...	....	....	...	7.3
Perfusion with ultrafiltrate from first 200 cc. sample, p_H 8.28	6.1	....	14.6	6.7*	7.0	....	5.9	....	8.4	...	11.1	....	9.4	5.8
	6.2	....	14.0	7.6	7.0	....	6.8	....	6.6	...	11.2	....	7.4	5.3
	6.0	....	....	8.0	6.4	....	6.3	....	6.0	...	10.6	....	7.4	5.6
	5.8	....	....	7.3	6.0	....	5.9	....	5.4	...	10.6	....	5.6	8.2
	5.3	....	....	...	5.8	....	5.4	....	5.1	...	9.8	....	4.2	7.8
	5.0	....	....	...	...	....	5.2	....	4.7	...	...	....	3.0	
Perfusion with buffered frog Ringer solution containing 0.4% sodium citrate, p_H 7.75	4.9	13.7	....	6.9	5.9	9.8	5.1	10.3	4.6	8.0	9.4	11.9	2.8	4.6
	5.0	13.0	....	6.4	6.0	11.2	4.8	11.0	5.0	8.7	9.3	11.5	3.0	4.6
	4.7	15.1	....	6.8	6.0	11.3	5.0	11.1	5.9	8.7	9.0	10.4	3.0	4.4
	5.2	15.4	....	7.6	6.3	11.7	5.0	11.0	5.9	9.3	9.4	10.3	2.8	4.6
	5.0	15.5	....	7.8	6.4	11.3	5.1	11.6	5.9	9.5	9.5	10.3	2.95	4.9
	5.0	16.5	....	8.0	6.4	11.5	5.1	10.6	5.9	9.5	9.8	10.1	2.95	
	5.0	15.2	....	...	6.4	....	5.1	10.4	5.8	9.3	10.2	....	3.0	
	5.0	17.0	....	...	6.5	....	5.2							
Ultrafiltrate from second 200 cc. sample, p_H 8.43	...	16.6	14.2	7.9†	...	10.7	...	10.6	...	8.7	....	10.4	4.2	
	...	14.4	12.8	7.8	...	10.2	...	9.4	...	6.9	....	8.4	4.5	
	...	....	12.7	7.0	...	10.6	...	9.0	...	5.5	....	8.6	4.2	
	...	....	11.8	6.5	...	10.0	...	8.4	...	5.2				
	...	....	‡	5.6	...	9.2	...	....	...	4.9				
										4.4				
Perfusion with buffered frog Ringer solution containing 0.4% sodium citrate	...	13.6	12.4	4.8	...	9.3	...	8.2	...	3.4	....	8.0		
	...	14.4	15.6	4.8	...	9.35	...	7.1	...	3.4	....	8.9		
	...	14.2	18.0	4.9	...	9.3	...	6.6	...	3.7	....	9.3		
	...	14.6	17.4	4.9	...	9.3	...	6.8	...	3.6	....	9.7		
	...	14.9	16.8	4.6	...	9.5	...	7.6	...	3.7	....	9.9		
	...	14.5	....	4.9	...	....	...	6.8	...	3.7	....	10.2		
	...	14.9	....	4.7	...	....	...	7.0	...	3.5	....	10.1		
								6.8	...	3.4				

* Ultrafiltrate 2.
† Ultrafiltrate 1.
‡ Temperature 30 below control.

III. Effect of Ultrafiltrates of Plasma from Patients Whose Blood Pressures Had Been Raised by Intravenous Injections of Angiotonin.—Table 9 presents certain pertinent data which should be consulted in the interpretation of the results presented in table 10, which records the findings in the perfusion of blood ultrafiltrates obtained from patients shown in table 9.

Study of the data in table 9 shows that intravenously administered angiotonin invariably pro-

duced a rise in blood pressure in the human subject. The amount of the elevation was fairly uniform. Seven to 10 cc. of angiotonin given intravenously, particularly in one dose, raised the blood pressure from the usual 120 systolic and 70 diastolic to 180 to 190 systolic and 110 six to eight minutes being the approximate duration of the elevation resulting from one injection. As previously stated, the specimens of blood were taken very rapidly with an evacuated bottle, in one to two minutes, at the peak of the elevation of blood pressure.

TABLE 9.—*Effects on Blood Pressures and the Duration of the Elevation of Blood Pressures of Intravenously Injected Angiotonin*

Patient	Date	Initial Blood Pressure	Blood Pressure Peak Due to Angiotonin	Amount of Angiotonin Injected Intravenously	Time for Blood Pressure Rise to Peak, Minutes	Duration of Rise, Minutes
B. C.	3/12/44	124/84	174/110	5 cc. in divided doses	9	16 (amebic dysentery)
A. J.	4/13/44	132/80	186/144	8.9 cc. in divided doses	12	20 (diabetes)
G. M.	4/17/44	136/94	180/116	7 cc. (in 5 and 2 cc. doses)	4	8
C. J.	5/ 6/44	130/68	210/140, 230/110	17 cc. (1 dose)	3	8
C. C. J.	5/ 9/44	120/80	200/114	10 cc. (1 dose)	4	9
J. C.	5/16/44	110/70	150 systolic	10 cc. (1 dose)	2	6
A. C.	5/19/44	122/72	193/110	15 cc. (1 dose)	3	7
A. D.	5/25/44	120/72	190/100	10 cc. (two 5 cc. doses)	4	7

TABLE 10.—*Effects on the Perfusion Rate of Ultrafiltrates of Blood Plasma from Patients with Normal Blood Pressures and from Blood Plasma Obtained During the Peak of the Elevation of Blood Pressure Due to Intravenously Injected Angiotonin*

Figures Represent Cubic Centimeters of Perfusate in Consecutive Two Minute Collection Intervals

Name.....	B. C.	G. C. M.	A. J.	C. J.	C. C. J.	J. C.	A. C.	A. C. D.
Blood pressure peak due to angiotonin intravenously	174/110	180/116	186/144	230/110	200/114	150/?	193/110	190/100
	(Each column represents the perfusion of a different frog)							
Perfusion rate with calcium-free citrated frog Ringer solution	4.2 4.8 4.4 4.4 4.6	4.7 5.9 5.4 5.2 4.6 5.2 6.0 4.9 5.5 4.9	1.4 5.0 1.5 3.0 1.7 2.0 1.6 2.0 1.5 1.8	11.0 9.6 13.0 10.3 14.0 10.3 14.2 10.6 14.7 10.7 14.6 10.8 11.8 12.6 11.0 11.4 12.6	16.2 9.2 16.0 10.0 16.4 10.1 16.4 10.0 16.4 9.8 16.6	10.2 18.0 11.0 18.2 11.1 18.0 .... 18.0 12.0 18.4 11.9 12.0	7.8 9.9 8.4 10.0 9.0 9.6 9.2 9.4 9.3 9.5 9.5 9.1 9.8 9.6	9.6 10.6 9.2 11.0 8.6 10.9 8.8 11.2 7.6 11.2 7.3 10.8 7.5 7.6
	A*	C A	C A	C A	C A	C A	C A	C A
Perfusion rate with ultrafiltrate from plasma obtained at peak of rise of blood pressure due to angiotonin	2.2 2.2 1.6 2.0 1.2	5.5 5.0 6.2 4.8 6.0 4.4 6.0 4.0 4.8 3.8	2.9 3.2 4.8 3.0 6.2 2.6 5.0 2.6 2.2 2.0 1.7 1.8 1.6 1.3	12.4 11.8 11.7 9.5 12.0 8.4 7.2 6.3 6.1	16.6 9.8 17.6 4.4 16.0 5.0 5.0 4.6 4.2	12.3 15.0 12.0 14.0 11.2 12.0 10.8	8.9 8.8 7.9 7.5 6.9 6.4 6.8 6.1 6.8 5.7 5.5	7.7 11.1 7.4 11.0 7.8 9.3 7.2 8.4 5.6 4.4
Perfusion rate with frog Ringer solution repeated	2.2 2.4 2.2 2.2 2.6 4.9 4.8 4.8 4.2 4.2 4.6	4.5 2.5 4.0 2.8 3.2 3.4 2.9 2.8 2.6 2.1	5.0 0.6 3.0 0.4 2.0 0.2 2.0 0.2 1.8	11.0 6.0 11.5 7.5 14.0 8.8 15.8 9.4 15.8 10.4 16.1 10.4 16.4 11.6 15.8 11.0 15.6 11.8 15.5 12.2 14.8 11.8 14.5 13.0 14.6	15.0 3.8 16.2 4.2 14.4 4.7 15.2 5.0 15.0 6.0 6.6 7.6 7.9 7.8 8.7 9.0 10.4 9.4	10.5 11.2 11.0 11.7 11.3 12.0 11.7 12.7 12.4 14.0 12.7 13.4 13.2 12.2 12.5	6.9 5.1 6.7 5.2 7.2 5.6 7.0 5.6 7.1 5.6 6.8 5.9 6.6 5.4 6.3 5.7 5.5 5.9	3.5 7.3 2.8 7.6 3.6 7.5 ... 7.8 3.8 8.0 3.6 8.0 3.6 7.4 3.9 7.4 4.0 6.8 6.6

* C indicates control plasma, 1st 200 cc. sample; A indicates angiotonin plasma, 2d 200 cc. sample, while blood pressure was elevated by angiotonin.

to 140 diastolic. The patient J. C. is an exception. Ten cubic centimeters of angiotonin raised his pressure from 110 systolic and 70 diastolic only to 150 systolic. The elevation in pressure is rapid, two to four minutes being required to reach the peak; and the fall is fairly rapid,

Table 10 contains the results of perfusing frogs with ultrafiltrates of the plasma from 8 patients in whom the blood pressure had been raised by intravenous injections of angiotonin. With the exception of patient B. C., each of the 7 others had two different bleedings of 200 cc. each

from the femoral artery. The first 200 cc. was taken immediately before and the second 200 cc. was taken during the peak of the rise in blood pressure due to intravenously administered angiotonin. Ultrafiltrates were made from the citrated plasma obtained from each bleeding. The effects on the perfusion rates of the two ultrafiltrates were noted by comparing the rates with those of ultrafiltrates from another group of normotensive patients in whom angiotonin was not injected.

The data in table 10 can be summarized by saying that perfusion of frogs with the ultrafiltrates from the plasma of the first 200 cc. control sample from 8 patients had a slight vasoconstrictor action. This has been previously called attention to in table 7, which contains the data on other patients with normal blood pressures. The slight decrease in rate of perfusion is attributed to vasoconstrictor effect, because the perfusion rate generally increased to or toward previous levels when the ultrafiltrates were followed by calcium-free citrated frog Ringer solution.

The ultrafiltrates from blood samples taken during the peak of the rise in blood pressure due to angiotonin, on the other hand, produced a decided drop in perfusion rates. This drop was manifested immediately and abruptly. The perfusion rate always returned toward or nearly to the preultrafiltrate rates when perfusion with calcium-free citrated frog Ringer solution followed perfusion with these ultrafiltrates.

Examination of the curves in the chart and of the tables which present data obtained from perfusion of ultrafiltrates of plasma from normal or hypertensive patients shows a considerable and abrupt fall in perfusion rates for the first two minute interval after the resumption of perfusion with calcium-free citrated frog Ringer solution. We are unable to explain this rather characteristic finding in the groups referred to. It may be due to technical factors or it may be due to sudden swelling of capillary endothelium owing to the difference in the composition of the two fluids. It is noteworthy that this fall did not occur after the perfusion of any of the fluids or ultrafiltrates containing angiotonin.

The chart shows graphically the results discussed. The sharp and extreme fall produced by perfusion of ultrafiltrates of blood taken at the peak of the rise in blood pressure due to angiotonin given intravenously is in pronounced contrast to the effects of plasma ultrafiltrates from normal and from hypertensive patients.

SUMMARY

A method for the bioassay of vasoconstrictor substances in ultrafiltrates of citrated plasma has been devised. With adequate control studies, ultrafiltrates of blood plasma from patients with normal blood pressure, with essential hypertension and with elevation of blood pressure produced by angiotonin have been bioassayed for vasoconstrictor substances. Ten tables and one graph presenting the results were prepared.

CONCLUSIONS

The vasoconstrictor effect of angiotonin can be detected in perfusions of whole pithed frogs.

Angiotonin may be ultrafiltrated and detected in blood plasma ultrafiltrates by this method of bioassay.

Angiotonin may be demonstrated in the ultrafiltrates of plasma from patients whose blood pressures have been elevated by intravenous injections of angiotonin.

Ultrafiltrates of blood plasma from normal and from hypertensive patients contain an almost identical amount of vasoconstrictor substance or substances.

Ultrafiltrates of blood plasma from patients made transiently hypertensive by angiotonin contain a much greater amount of vasoconstrictor substance than ultrafiltrates of plasma from hypertensive patients whose blood pressures are much higher.

Plotted curves of vasoconstrictor effects of ultrafiltrates of plasma from normotensive and from hypertensive persons are identical and differ greatly in character from curves of vasoconstrictor effects of angiotonin in frog Ringer solution or in ultrafiltrates of blood plasma to which angiotonin has been added.

Angiotonin is not present in increased amounts in the blood of patients with essential hypertension of long duration.

In essential hypertension of long duration, the elevation of blood pressure is not due to increased production of angiotonin. Essential hypertension is probably not caused by an increased production of angiotonin.

OPTIMUM DOSE OF SULFADIAZINE IN TREATMENT OF PNEUMOCOCCIC PNEUMONIA

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Extensive experience with the sulfonamide drugs in clinics throughout the United States has established sulfadiazine as the drug of choice in the treatment of pneumococcic pneumonia because of its greater efficacy and lower toxicity.[1] Hence it is important to establish its optimum dose in order that it may be used with maximum effectiveness.

The initial dose of sulfadiazine usually advocated is 4 or 5 Gm.[2] However, it varies from 2[3] to 7[11] Gm. A maintenance dose of 1 Gm. every four hours is usually given,[4] although 1 Gm.[5] or 2 Gm.[11] every six hours have also been used. It has been demonstrated[11] that, since sulfadiazine is excreted slowly, a proportionately higher dose given every six hours is as effective in maintaining adequate blood concentrations as is a four hourly dose.

Early in the use of sulfadiazine, its administration by the intravenous route was reserved for critically ill patients and for patients who could not take medicaments[6] orally. More recently, however, the administration of 5 Gm. of sodium sulfadiazine intravenously as soon as the diagnosis of pneumococcic pneumonia is made has been recommended for all patients who are moderately or severely ill. This is to be repeated

Martha C. Eaton, A.B., aided in compiling statistical data.

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2. (*a*) Volini, I. F.; Engbring, G.; Peffer, J. R., and Schorsch, H.: The Treatment of Pneumonia with Sulfadiazine: Five Hundred and Thirty-Three Patients in Two Seasons, M. Clin. North America **28**:267 (Jan.) 1944. (*b*) Finland, Strauss and Peterson.[1b] Dowling and others.[1c] Shackman and Bullowa.[1j] Peterson and Finland.[1l] Dowling and others.[1n] Samper and Finland.[1o]

3. (*a*) Jeck, H. S., and Orkin, L.: Toxic Renal Reactions Following the Use of Sulfadiazine, J. Urol. **49**:290 (Feb.) 1943. (*b*) Dowling and others.[1c] Dowling and others.[1n]

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5. Flippin, Schwartz and Domm.[1g] Shackman and Bullowa.[1j]

6. Flippin and others.[1a] Finland, Strauss and Peterson.[1b] Dowling and others.[1c] Flippin, Schwartz and Domm.[1g] Shackman and Bullowa.[1j] Billings and Wood.[4a]

if, for any reason, adequate dosage cannot be maintained by oral medication.[7] It has been pointed out that intravenous administration of the initial dose starts therapy at once,[8] avoiding the delay of four to eight hours required for the absorption of sulfadiazine given orally.[9] Peterson and Finland [11] stated that sulfadiazine is erratically absorbed in sick and toxic patients and that therapeutic levels may be difficult to reach in such patients by oral medication alone.

Blood concentrations of sulfadiazine with usual doses vary from 5 to 10 mg. per hundred cubic centimeters.[10] It has been recommended that the blood level be maintained between 8 and 12 mg.,[11] 8 and 15 mg.[11] or 8 and 10 mg.[2a] and at no less than 10 mg. in moderately ill patients and between 15 and 20 mg. in severely ill patients.[11]

Although Dowling and his associates [12] entitled their study "The Comparative Value of High and Low Doses of Sulfadiazine in the Treatment of Pneumococcic Pneumonia," they actually compared usual doses with low doses. They did not use more than the usual dose (4 to 5 Gm. initially and 1 Gm. every four hours thereafter) at any time. The low dose consisted of 2 Gm. initially and 0.5 Gm. every four hours thereafter. They found no significant difference between the mortality rates in these two groups. The group receiving the lower dose had a higher morbidity and had almost 50 per cent more complications than the group receiving the higher dose. The only conclusion that can be drawn from this study is that doses smaller than the usual are not so effective.

Frisch, Price and Myers [7a] found that lower than usual doses for mildly ill patients with pneumonia, having sputum counts of less than 35 pneumococci per oil immersion field, resulted in a higher incidence of relapses and complications.

Marangoni and D'Agati,[13] in treating meningococcic infections, gave 25 Gm. of sulfadiazine during the first twenty-four hours and 12 to 15 Gm. daily thereafter, maintaining blood levels of from 15 to 20 mg. per hundred cubic centimeters. By such a regimen they obtained a mortality rate among the patients thus treated of only 3.1 per cent. After contrasting their results with the usual 10 per cent mortality rate reported in the literature, they recommended the use of massive doses of sulfadiazine in the treatment of meningococcic infections.

Browne [4b] noted 1 death in 100 consecutive patients with pneumonia who obtained high blood levels of sulfadiazine from 8 to 30.8 mg. per hundred cubic centimeters. Frisch, Price and Myers [7a] reported on a group of patients (classed as severely ill) with sputum counts between 36 and 50 pneumococci per oil immersion field who were given double the routine dose (4 Gm. intravenously and 4 Gm. orally, then 2 Gm. every four hours thereafter). The mortality rate in this unfavorable group, only 9 per cent, they ascribed to the use of "intensive chemotherapy." Patients classed by them as having "overwhelming pneumonia," with sputum counts over 50 per oil immersion field, who received the usual dose (4 Gm. initially followed by 1 Gm. every four hours) had a mortality rate of 79 per cent. In a similar group who received double this dose the mortality rate was 42 per cent. The conclusion reached by these investigators was that "massive doses of sulfonamide intravenously offer a greater chance of survival" and that "it is possible even larger doses might be more effective when the sputum count exceeds 50 per field."

In a group of patients previously reported on [11] it was found that the maintenance of higher sulfadiazine blood levels reduced the gross mortality. In that study it was frequently observed that patients would have large numbers of pneumococci in the sputum and active spread of

7. (*a*) Frisch, A. W.; Price, A. E., and Myers, G. B.: Pneumococcic Pneumonia: The Selection and Control of Serum and Chemotherapy by Sputum Examinations, Am. J. M. Sc. **205**:771 (June) 1943. (*b*) Collen and Dybdahl.[11] Shackman and Bullowa.[1j] Peterson and Finland.[11]

8. Collen and Dybdahl.[11] Volini and others.[2a] Frisch, Price and Myers.[7a]

9. (*a*) Reinhold, J. G.; Flippin, H. F.; Schwartz, L., and Domm, A. H.: The Absorption, Distribution, and Excretion of 2-Sulfanilamido Pyrimidine (Sulfapyrimidine, Sulfadiazine) in Man, Am. J. M. Sc. **201**:106 (Jan.) 1941. (*b*) Peterson, O. L.; Strauss, E.; Taylor, F. H. L., and Finland, M.: Absorption, Excretion and Distribution of Sulfadiazine (2-Sulfanilamido-Pyrimidine), ibid. **201**:357 (March) 1941. (*c*) Samper and Finland.[10]

10. Flippin and others.[1a] Dowling and others.[1c] Finland, Peterson and Goodwin.[1e] Shackman and Bullowa.[1j] Dowling and others.[1n] Samper and Finland.[10] Volini and others.[2a] Jeck and Orkin.[3a] Reinhold and others.[9a] Peterson and others.[9b]

11. Lowell, F. C.: The Present Status of Sulfonamide Therapy, M. Clin. North America **27**:1247 (Sept.) 1943.

12. Dowling, H. F.; Hartman, C. R.; Feldman, H. A., and Jenkins, F. A.: The Comparative Value of High and Low Doses of Sulfadiazine in the Treatment of Pneumococcic Pneumonia, Am. J. M. Sc. **205**:197 (Feb.) 1943.

13. Marangoni, B. A., and D'Agati, V. C.: Treatment of 134 Cases of Meningococcic Infection with Massive Doses of Sulfadiazine, Am. J. M. Sc. **207**:67 (Jan.) 1944.

pneumonia with the usual doses of sulfadiazine but would show prompt improvement with an increase in the dosage of the drug. This observation prompted the present clinical study.

Gross mortality statistics for pneumonia have little comparative value, since so many variable factors are involved in determining the severity of pneumonia in an individual patient. No 2 cases or series of cases can be directly compared unless they represent conditions of approximately equal severity.

The mortality rates of pneumococcic pneumonia treated with sulfadiazine and specific antiserums in large series of reported cases vary from 7.6 per cent (Billings and Wood [4a] in a series containing fewer patients with bacteremia and more patients treated early in the course of the disease) to 14.3 per cent (Shackman and Bullowa [3j] in a series containing a higher percentage of older patients). The average mortality rate with sulfadiazine therapy in the larger series reported is approximately 10 per cent.[14]

Because the series reviewed does not necessarily represent cases of pneumonia of equal severity, the gross statistics are of value only in a general way in indicating success of treatment.

OUTLINE OF CLINICAL STUDY

It is the purpose of this study to compare results for two series of patients, in whom the disease was similarly severe, cared for in the same hospital by the same staff and treated with the same general therapeutic routine, except that the first group received the usual doses of sulfadiazine while all moderately and severely ill patients in the second group received double the usual dose.

The diagnosis of pneumonia was substantiated in every case by positive roentgenographic evidence. No patients with "minimal pneumonia," "pneumonitis" or similar indefinite diagnoses were included in either series. Patients with pneumonia as a contributory diagnosis to another primary illness were excluded.

From September 1942 through August 1943, 618 patients with pneumococcic pneumonia were treated at the Permanente Foundation Hospital. The majority of this group received 4 or 5 Gm. of sulfadiazine orally as an initial dose; severely ill patients received an initial dose of 5 Gm. of sodium sulfadiazine intravenously and 2 Gm. of sulfadiazine orally. One gram of sulfadiazine every four hours or 2 Gm. every six hours was administered as a maintenance dose. Average blood levels varied from 8 to 10 mg. of sulfadiazine per hundred cubic centimeters. As is evident, this first group received the usually recommended dose of sulfadiazine.

Adjuvant therapy in the form of fluids, expectorant cough mixtures, oxygen by inhalation and specific pneumococcus serum (see table 9) was used routinely. In this group of 618 patients 66, or 10.7, per cent died.

From September 1943 through April 1944, a group of 748 patients with pneumococcic pneumonia were treated at this hospital. This group of patients received the same adjuvant therapy and general therapeutic regimen as the first group. However, every patient in this second group who was moderately or severely ill received 5 Gm. of sodium sulfadiazine intravenously and 5 Gm. of sulfadiazine orally (total 10 Gm.) as an initial dose. This was followed by 4 Gm. of sulfadiazine orally every six hours as a maintenance dose. In this group blood sulfadiazine levels were found to range from 12 to 20 mg. per hundred cubic centimeters. In critically ill patients attempts were made to maintain blood sulfadiazine levels at about 20 mg. or even higher. In this second group (748 patients) 46, or 6.2 per cent, died.

These results indicate that for patients with pneumococcic pneumonia higher doses of sulfadiazine (10 Gm. initially and 4 Gm. every six hours thereafter, producing blood levels of sulfadiazine of 12 to 20 mg. per hundred cubic centimeters) resulted in a gross mortality rate of only 6.2 per cent, as compared with a mortality rate of 10.7 per cent for those treated with the usual dosage (average 5 Gm. initially and 2 Gm. every six hours thereafter, with blood levels of sulfadiazine of 8 to 10 mg.). With no other change in therapy, doubling the dose of sulfadiazine decreased the gross fatality rate by 40 per cent of its previous figure. This confirms the report of Frisch, Price and Myers.[7a]

COMPARATIVE SEVERITY OF ILLNESS IN THE TWO SERIES

Since it has been emphasized that gross mortality statistics have little comparative value unless they are for illnesses of approximately equal severity, these two groups are analyzed

14. (*a*) Collen, M. F.; Dybdahl, G. L., and O'Brien, G. F.: A Study of Pneumonia in the Shipbuilding Industry: The Epidemiology and Management of 864 Cases over a One-Year Period in the Kaiser Richmond Shipyards, J. Indust. Hyg. & Toxicol. **26**:1 (Jan.) 1944. (*b*) Flippin and others.[1a] Finland, Strauss and Peterson.[1b] Dowling and others.[1c] Smith.[1d] Finland, Peterson and Goodwin.[1e] Bortz.[1f] Flippin, Schwartz and Domm.[1g] Dowling and Lepper.[1h] Reimann.[1k] Dowling and others.[1n] Volini and others.[2a]

as to the comparative severity of the various important factors which influence the mortality rate of pneumococcic pneumonia (tables 1 through 9).

Age of Patients.—As is demonstrated in table 1, the age of a patient is an extremely important factor influencing mortality in pneumonia. Table 1 further shows that the comparative incidence according to age agrees very closely in the two series. It is important to note that 51 per cent of the patients in both series were over 40 years of age and that only 6 per cent were under 20 years of age. It is of interest that in the series of patients receiving the double dose the mortality rate is lower in every decade.

Comparative Severity According to Number of Lobes Involved.—The extent of involvement of the lungs by pneumonia is of great importance in determining the severity of the disease, as is evident in table 2. The incidence of multiple lobar involvement is slightly higher in the group receiving the double dose. This factor tends to favor the group receiving the usual dose as being less severely ill on the whole. However, the double dose regimen was as effective in lowering the mortality rate in the group with multiple lobar involvement as it was in those patients with involvement of only a single lobe.

TABLE 1.—*Comparative Incidence by Age*

Age, Years	Usual Dose				Double Dose			
	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent
Under 20	35	5.7	1	2.9	49	6.6	..	0.0
20-29	129	20.9	5	3.9	141	18.8	3	2.1
30-39	137	22.1	9	6.6	177	23.7	5	2.8
40-49	136	22.0	25	18.4	172	22.9	10	5.8
50-59	103	16.7	16	15.5	126	16.9	18	14.3
60-69	72	11.6	10	13.9	75	10.0	9	12.0
70 and over	6	1.0	0	....	8	1.1	1	....
Total	618	100.0	66	10.7	748	100.0	46	6.2

TABLE 2.—*Comparative Severity by Number of Lobes Involved*

Involvement, Lobe	Usual Dose				Double Dose			
	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent
Single	438	70.9	22	5.0	498	66.5	16	3.2
Multiple	180	29.1	44	20.5	250	33.5	30	12.0
Total	618	100.0	66	10.7	748	100.0	46	6.2

TABLE 3.—*Comparative Incidence of Specific Pneumococcic Types*

Pneumococcic Type	Usual Dose				Pneumococcic Type	Double Dose			
	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent		Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent
VII	68	11.0	20	29.4	VII	92	12.3	10	10.9
IV	34	5.5	5	14.7	XXV	55	7.4	1	1.8
I	29	4.7	4	13.8	I	52	7.0	6	11.5
XXV	26	4.2	3	11.5	III	47	6.3	16	34.0
II	22	3.6	6	27.3	V	45	6.0	2	4.4
VIII	16	2.6	0	0.0	VIII	32	4.3	0	0.0
XII	14	2.3	2	14.3	IV	31	4.1	4	12.9
III	11	1.8	2	18.2	XII	30	4.0	2	6.7
V	11	1.8	2	18.2	XXIV	13	1.7	1	7.7
IX	10	1.6	2	20.0	XXXII	13	1.7	0	0.0
XX	9	1.5	1		XX	11	1.5	0	
XXXIII	8	1.3	1		XXXIII	11	1.5	0	
XIV	7	1.1	1		II	10	1.3	2	
VI	6	1.0	1		XVIII	10	1.3	0	
XXIV	5	0.8	0		XIX	10	1.3	1	
Organisms typed for 311 patients. Total patients, 618					Organisms typed for 532 patients. Total patients, 748				

Comparative Incidence of Specific Pneumococcic Types.—Table 3 reveals that Pneumococcus type VII was in both series the most frequent etiologic organism and was encountered with approximately the same incidence. Types XXV and I were also prominent in both groups. It is important to note that illnesses caused by type III not only greatly increased in frequency in the group receiving the double dose, but almost doubled in percentage of mor-

tality. This observation suggests that sulfadiazine in any dosage is of little value in the treatment of Pneumococcus type III pneumonia. Although it is difficult to draw any conclusions from table 3, it is apparent that the same types were prominent in both groups and that a general decrease in percentage of mortality occurred in the group receiving the double dose (exclusive of type III).

Comparative Incidence of Bacteremia.—Blood for culture was taken routinely on every patient before any sulfadiazine was administered. The presence of bacteremia is an important factor influencing the mortality of pneumococcic pneumonia, as demonstrated in table 4. This table shows that the incidence of bacteremia was almost identical in the two groups of patients. In patients with a positive blood culture, a great decrease in mortality rate occurred in the group treated with the double dose regimen as compared with those treated with usual doses.

Comparative Incidence of Leukopenia.—The importance of leukopenia as a prognostic factor in pneumococcic pneumonia[14a] is not always emphasized, but, as is clearly demonstrated in table 5, a leukocyte count below 6,000 cells per cubic millimeter is a grave sign. Leukocyte counts presented in this table are those taken on the patient's admission to the hospital and cannot be interpreted as being due to sulfadiazine toxicity. (Leukopenia as a manifestation of drug toxicity is presented in table 12.) Table 5 demonstrates that the incidence of leukopenia is identical in the two groups of patients. In the group of patients critically ill with leukopenia, the double dose regimen resulted in a considerably decreased mortality.

TABLE 4.—*Comparative Incidence of Bacteremia*

	Usual Dose				Double Dose			
Bacteremia	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent
Present	76	12.3	25	32.9	93	12.4	15	16.1
Absent	542	87.7	41	7.7	625	83.6	31	5.0
Unknown or not determined	0	0.0	0	0.0	30	4.0	0	0.0
Total	618	100.0	66	10.7	748	100.0	46	6.2

TABLE 5.—*Comparative Incidence of Leukopenia*

	Usual Dose				Double Dose			
Leukocytes per Cu. Mm.	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent
Under 6,000	34	5.5	22	66.7	42	5.6	20	47.6
6,000 to 10,000	72	11.6	13	18.3	94	12.7	9	9.6
10,000 to 25,000	376	60.9	22	5.9	455	60.9	13	2.9
Over 25,000	127	20.5	9	7.1	153	20.3	3	2.0
Unknown	9	1.5	0	...	4	0.5	1	...
Total	618	100.0	66	10.7	748	100.0	46	6.2

TABLE 6.—*Comparison of Number of Days of Illness Before Hospitalization*

	Usual Dose				Double Dose			
Days of Illness, Number	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent
1	156	25.2	12	7.7	187	25.0	6	3.2
2	145	23.5	14	9.7	138	18.3	3	2.2
3 or 4	164	26.6	20	12.2	206	27.7	11	5.3
Over 4	114	18.4	15	13.2	202	27.0	24	11.9
Unknown	39	6.3	5	....	15	2.0	2	....
Total	618	100.0	66	10.7	748	100.0	46	6.2

Comparison of Number of Days of Illness Prior to Hospitalization.—The number of days a patient is ill before his admittance to the hospital is of some importance in influencing the mortality of pneumococcic pneumonia. The onset of illness was considered to be the first day on which the patient noted pain in the chest, blood-streaked sputum or chill. The incidence of patients admitted after only one day of illness is the same in both groups, as shown in table 6. However, the group receiving the double dose contained a greater number of patients who were admitted late in their illness. This factor tends

to favor the group receiving the usual dose as being less severely ill.

Comparative Incidence by Severity of Associated Diseases.—Obviously the presence of an associated disease may be highly important in influencing the mortality of pneumonia. The grading of the severity of an associated disease is difficult and depends on the judgment of the examiner. Some uniformity of grading was attempted by grading an associated disease as "mild" if it was sufficienty severe to aggravate or alter the course of the pneumonia, "moderate" if it was sufficiently severe so that associated with pneumonia it might endanger the life of the patient and "severe" if the associated disease was in itself sufficient to jeopardize the life of the patient. Table 7 shows that associated diseases were present with the same frequency in the two groups. Associated diseases graded as "severe" were present in 17 per cent of both series.

TABLE 7.—*Comparative Incidence by Severity of Associated Diseases*

Associated Diseases, Grade of Severity	Usual Dose				Double Dose			
	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent	Patients, Number	Incidence, per Cent	Deaths, Number	Mortality, per Cent
Present	122	19.7	17	13.9	142	19.0	15	10.6
Mild	64	52.5	4	6.2	34	36.6	2	5.9
Moderate	37	30.3	4	10.8	43	46.2	0	0.0
Severe	21	17.2	9	42.8	16	17.2	12	75.0
Total graded	122	100.0	17	13.9	93	100.0	14	15.1
Severity not graded	0	0.0	0	0.0	49	0.0	1	2.0
Not present	496	80.3	49	9.9	606	81.0	31	5.1
Total	618	100.0	66	10.7	748	100.0	46	6.2

TABLE 8.—*Comparative Number of Deaths Occurring Within Six Hours After Admission*

Time of Death, Hours After Admission	Usual Dose		Double Dose	
	Deaths, Number	Deaths, per Cent	Deaths, Number	Deaths, per Cent
Under 6	3	4.5	5	10.9
Over 6	63	95.5	41	89.1
Total	66	100.0	46	100.0

Comparative Number of Deaths Occurring Within Six Hours After Patient's Admission.—Patients who die within a few hours after their admission to the hospital obviously alter mortality statistics without indicating the effect of therapy. Therefore it is important to consider this factor in comparing these two series. Table 8 indicates that over twice as many patients who died within six hours were admitted during the period when the double dose regimen was used than when the usual dose of sulfadiazine was used. By eliminating patients who died within six hours after their admission, the mortality rate of the patients treated with the usual dose is 10.2 per cent and with the double dose, 5.5 per cent.

Frequency of Use of Adjuvant Specific Pneumococcal Therapy.—Adjuvant therapy, as outlined elsewhere,[11] was identical in both groups. Specific pneumococcus serum was given throughout for similar indications. Five and eight-tenths per cent more patients in the group receiving the double dose received specific serum than in the group receiving the usual dose, as shown in table 9. This increased use of serum was primarily due to its liberal administration to the majority (59.6 per cent) of patients with Pneumococcus type III pneumonia. In all other types, the frequency with which adjuvant serum was used differed in the two groups by only 3.7 per cent. There is no reason to believe that this small increase in the use of serum could influence the final mortality statistics to any great degree, because it has been shown that when an adequate dosage of sulfadiazine is used little further decrease in mortality occurs with the added use of specific serum.[14a] The increased use of serum was as ineffectual as the increased dose of sulfadiazine (table 3) in reducing the mortality of type III Pneumococcus pneumonia.

TABLE 9.—*Comparative Frequency of Use of Adjuvant Serum Therapy*

Serum	Usual Dose		Double Dose		
	Patients, Number	Incidence, per Cent	Patients, Number	Incidence, per Cent	Increase, per Cent
Type III					
Serum used	5	45.5	28	59.6	14.1
Serum not used	6	54.5	19	40.4	....
Others (exclusive of III)					
Serum used	88	14.5	128	18.2	3.7
Serum not used	519	85.5	573	81.8	...
All types					
Serum used	93	15.1	156	20.9	5.8
Serum not used	525	84.9	592	79.1	...
Total	618	100.0	748	100.0	

14a. Frisch, Price and Myers.[7a] Plummer, N., and Others: Chemotherapy Versus Combined Chemotherapy and Serum in the Treatment of Pneumonia: A Study of 607 Alternated Cases, J. A. M. A. **116**: 2366 (May 24) 1941.

Summary of Factors of Comparative Severity.—Tables 1, 3, 4, 5 and 7 demonstrate that the two groups of patients are comparable and similar with reference to (1) size, (2) age incidence, (3) comparative incidence of specific pneumococcus types, (4) incidence of bacteremia, (5) incidence of leukopenia and (6) comparative severity of associated diseases.

Tables 2, 6 and 8 show that the group receiving the double dose could be considered somewhat more severely ill because of (1) a higher incidence of multilobar involvement, (2) a greater number of patients admitted late in their illness and (3) a greater incidence of patients in terminal stages who died within six hours after admission. On the other hand, table 9 indicates that this group was favored by a slightly higher frequency with which adjuvant specific pneumococcus serum was used. In general these two groups of patients are remarkably similar with reference to the various factors which influence mortality in pneumococcic pneumonia. Although there are a few factors which tend to favor each group, it is reasonable and justifiable to assume that they tend to balance and will not be of great importance in influencing the final results.

It has been established that these two groups of patients are approximately equal in severity of illness, and therefore their mortality statistics can be directly compared.

THERAPEUTIC EVALUATION OF USUAL AND DOUBLE DOSES OF SULFADIAZINE

The evaluation of the success of therapy in pneumonia encompasses a study of the mortality rate, the incidence of complicating conditions, the length of stay in the hospital and the frequency of relapse.

Gross Mortality Rate.—The data presented here demonstrated that in two groups of patients with pneumonia of comparable severity the gross mortality in those treated with usual doses of sulfadiazine was 10.7 per cent, while in those treated with double the usual dose it was 6.2 per cent. The corrected mortality rate, excluding patients who died within six hours after their admission to the hospital, was 10.2 per cent with the usual dose and 5.5 per cent with the double dose.

Comparative Incidence of Complicating Conditions.—Table 10 shows that the incidence of sterile pleural effusions in the group of patients treated with the double dose regimen was only 2.7 per cent, as compared with 5.2 per cent in the group treated with usual doses. The incidence of other important complicating conditions was equally low in both series.

Comparative Length of Stay in the Hospital.—Excluding those who died, 65.8 per cent of patients treated with double doses had recovered from their illness and were discharged from the hospital in less than seven days, whereas only 45.0 per cent of patients treated with usual doses had a similar stay. The criteria for discharge in both groups were the same: namely, chemotherapy was discontinued after twenty-four to forty-eight hours of normal temperature, and the average patient was discharged twenty-four to forty-eight hours later. Severely ill patients usually required two to four more days of convalescence. It is also noteworthy that only 8.1 per cent of patients treated with double doses required over two weeks of hospitalization, whereas 14.4 per cent of patients treated with usual doses required over fourteen days of hospital care.

TABLE 10.—*Comparative Incidence of Complicating Conditions*

Complication	Usual Dose		Double Dose	
	Patients, Number	Incidence, per Cent	Patients, Number	Incidence, per Cent
Sterile effusion	32	5.2	20	2.7
Empyema	1	0.2	2	0.3
Pulmonary abscess	1	0.2	4	0.6
Meningitis	2	0.3	0	0.0
Endocarditis	1	0.2	2	0.3
Pericarditis	1	0.2	2	0.3

TABLE 11.—*Comparative Length of Hospital Stay* *

Hospital Days	Usual Dose		Double Dose	
	Patients, Number	Cases, per Cent	Patients, Number	Cases, per Cent
Under 7	248	45.0	461	65.8
7 to 14	224	40.6	184	26.1
15 to 21	40	7.2	29	4.1
22 to 28	17	3.1	11	1.6
Over 28	23	4.1	17	2.4
Total	552	100.0	702	100.0

* Fatalities are excluded from these figures.

Reliable data on the incidence of relapses are not available on the group of patients treated with usual doses, and so no comparison can be drawn.

The treatment of patients with pneumococcic pneumonia with double doses of sulfadiazine resulted in (1) a decrease in the mortality rate from 10.2 to 5.5 per cent, (2) a decrease in the incidence of pleural effusions from 5.2 to 2.7 per cent and (3) a decrease in the average

number of days in the hospital required for recovery (66 per cent required less than seven days, as compared with 45 per cent).

INCIDENCE OF SULFADIAZINE TOXICITY

It is important in studying the use of any drug to evaluate its toxicity. Numerous investigators have demonstrated that there are two distinct types of toxic reactions to sulfadiazine. The control of the commoner type and the prevention of such conditions as crystalluria and hematuria depend on an adequate volume of urine of sufficient alkalinity to dissolve the sulfadiazine. The problem can be easily eliminated by proper administration of alkali.[15] Every patient on the double dose regimen received the initial 5 Gm. of sodium sulfadiazine intravenously in 500 cc. of sixth-molar sodium lactate solution. (It is noteworthy that to maintain sodium sulfadiazine in solution, the p_H of sixth-molar sodium lactate solution should be 7 to 7.2.) Five grams of sodium bicarbonate was administered orally every six hours while the patient was receiving sulfadiazine. The decreased frequency of crystalluria in the group receiving the double dose was dependent on the routine use of alkali (table 12). The incidence of crystalluria and/or hematuria in this group was less than that reported by some authors[16] who used usual doses of sulfadiazine without administering alkali.

Reactions, listed in table 12, which are direct manifestations of sensitivity to sulfadiazine show no remarkable difference in frequency in the two groups. It is noteworthy that the incidence of the sensitivity manifestations of sulfadiazine toxicity listed in table 12 are comparable to those of other large series reported.[17]

SUMMARY

In a twenty month period 1,465 patients with pneumococcic pneumonia were treated at the Permanente Foundation Hospital. In the first half of this period 618 consecutive patients were treated with the usual doses of sulfadiazine, namely, 5 Gm. initially and 2 Gm. every six hours thereafter, maintaining an average blood level of 8 to 10 mg. per hundred cubic centimeters. In the second half of this period 748 consecutive patients with pneumococcic pneumonia were treated with double doses of sulfadiazine. The majority of patients in the group receiving the double dose received 5 Gm. of sodium sulfadiazine in 500 cc. of sixth-molar sodium lactate solution (p_H 7.0 to 7.2) intravenously and 5 Gm. of sulfadiazine orally (total 10 Gm.) initially, followed by 4 Gm. of sulfadiazine orally every six hours thereafter, maintaining average blood concentrations of sulfadiazine from 12 to 20 mg.

A comparative analysis of the various important factors influencing the mortality rate in pneumococcic pneumonia was made, with special reference to age, specific pneumococcic types, incidence of bacteremia, leukopenia, severity of associated diseases, extent of pneumococcic involvement, number of days of illness

TABLE 12.—*Comparative Incidence of Sulfadiazine Toxicity*

Toxic Reaction	Usual Dose		Double Dose	
	Reactions, Number	Incidence, per Cent	Reactions, Number	Incidence, per Cent
Crystalluria and/or hematuria	84	13.6	53	7.1
Cutaneous rash	10	1.6	10	1.3
Psychosis	7	1.1	7	0.9
Fever	2	0.3	3	0.4
Leukopenia or agranulocytosis	2	0.3	0	0.0
Hepatitis	0	0.0	1	0.1
Total (excluding urinary reactions)	21	3.4	21	2.8

15. Jensen, J. O., Jr., and Fox, C. L., Jr.: Hydrogen Ion Concentration and the Solubility of Sulfonamides in Urine: The Relation to Renal Precipitation, J. Urol. **49**:334 (Feb.) 1943. Gilligan, D. R.; Garb, S., and Plummer, N.: Prevention of Crystalluria During Sulfadiazine Therapy: Experimental and Clinical Studies, Proc. Soc. Exper. Biol. & Med. **52**:248 (March) 1943. Hall, W., and Spink, W., cited by Watson, C. J., in discussion on Murphy, F. S.; Kuzma, J. F.; Polley, T. Z., and Grill, J.: Renal Damage Due to Sulfonamides, J. A. M. A. **124**:800 (March 18) 1943. Fox, C. L., Jr.; Jensen, O. L., Jr., and Mudge, G. H.: Prevention of Renal Obstruction During Sulfadiazine Therapy, ibid. **121**:1147 (April 3) 1943. Gilligan, D. R.; Garb, S.; Wheeler, C., and Plummer, N.: Adjuvant Alkali Therapy in Prevention of Renal Complications of Sulfadiazine, ibid. **122**:1160 (Aug. 21) 1943. Gilligan, D. R.; Dingwall, J. A., III, and McDermott, W.: The Parenteral Use of Sodium Lactate Solution in the Prevention of Renal Complications from Parenterally Admnistered Sodium Sulfadiazine, Ann. Int. Med. **20**: 604 (April) 1944. Rohr, J. H., and Christopher, F.: Administration of Alkalis in Sulfadiazine Therapy, Surg., Gynec. & Obst. **78**:515 (May) 1944. Ohnysty, J., and Wolfson, W. Q.: Potassium Bicarbonate: An Adjunct to Chemotherapy in Pneumonia Complicating Cardiac Decompensation, New England J. Med. **231**: 381 (Sept. 14) 1944. Plummer and Wheeler.[1m]

16. Finland, Peterson and Goodwin.[1o] Volini and others.[2a]

17. Long, P. H.: Sulfonamide Compounds in the Prevention and Treatment of Wound Infection, J. A. M. A. **121**:303 (Jan. 30) 1943. Finland, Peterson and Goodwin.[1o] Flippin, Schwartz and Domm.[1g] Shackman and Bullowa.[1j] Dowling and others.[1n] Volini and others.[2a]

prior to hospitalization and incidence of patients admitted who died soon after. It was found that these two groups of patients had pneumococcic pneumonia of comparable severity. Adjuvant therapy, including the use of specific pneumococcus serum, was similar in both groups.

The only significant difference in these two groups of patients was that one group was treated with approximately twice the usual dose of sulfadiazine.

The treatment of patients with pneumococcic pneumonia with double doses of sulfadiazine resulted in (1) a decrease in the gross mortality from 10.7 to 6.2 per cent (corrected for patients dying within six hours after their hospitalization, the mortality rate decreased from 10.2 to 5.5 per cent), (2) a decrease in the incidence of sterile pleural effusions from 5.2 to 2.7 per cent and (3) a decrease in the average number of days in the hospital required for recovery (66 per cent required less than seven days, as compared to 45 per cent).

There was no greater incidence of drug toxicity in patients treated with double doses of sulfadiazine than in those treated with usual doses.

CONCLUSIONS

A study of two large groups of patients with pneumococcic pneumonia of comparable severity, treated similarly in every respect except that one group received double doses of sulfadiazine, demonstrated that the use of higher doses of sulfadiazine resulted in a great decrease in mortality rate, and a decrease in length of stay in the hospital, without any notable change in incidence of drug toxicity.

For severely ill adult patients with pneumococcic pneumonia, sufficiently large doses of sulfadiazine should be administered to maintain blood concentrations of at least 15 mg., with the optimum blood levels maintained preferably at about 20 mg. of sulfadiazine per hundred cubic centimeters.

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REVERSAL OF LINGUAL ATROPHIC CHANGES WITH NICOTINAMIDE THERAPY

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Description by Kruse [1] (1942) of the changes in the tongues of a group of adults which were reversed toward normal by the daily administration of 200 mg. of nicotinamide led us to make an attempt to establish the validity of these conclusions in the prison population which we have been studying. The description of the diet used by these men has been given by Kyhos and associates [2] (1944), and it suggested that extreme deficiencies of nicotinic acid were not to be expected. The amounts of meat served and eaten were variable but never large. The bread used was baked in the prison and, so far as we can learn, was not made from enriched flour. The diet was not too different from what is used by a large part of the population engaged in industry or in agriculture, and therefore the observations may well be applicable to the medical evaluation of nutritional status of patients seen in any clinic.

As a first step, 102 men were selected at random and, without any warning, asked to stop work in the prison shops long enough to have their tongues inspected. None of these men were volunteers in the other experimental procedures we were engaged in at the time. From this inspection we recorded data on 30 men showing obvious changes such as Kruse has described. At the time of the first study a biomicroscope was not available. Hence all these observations are based on gross inspection only. The changes which we have studied have been listed as fissures, atrophy of filiform papillae and dental scalloping of the margin of the tongue. We did not see any of the evidences of acute pellagra. According to Kruse's classification, our subjects would probably be listed as being in stages three and four of chronic aniacinosis

From among this group of 30 prisoners we were able, on request, to secure some cooperation from 23 men. They were given a daily dose of 50 mg. of nicotinamide, administered and taken in the presence of one of the guards to make sure of its ingestion. The dose was maintained for a period of nineteen weeks, and observations were repeated by the same observer at the end of four, twelve and nineteen weeks. Subjective comments told of improved appetite or sense of vigor in a number of cases, but these were not consistent. A few men noted small gains in weight. There were no ill effects noted. The men knew only that they were being given pure vitamins. No other change in routine was called for.

Of the 23 men, there were 3 in whose tongues no fissures or crevices were observed. Of the remaining 20, with easily visible fissures, 3 could not be followed consistently enough to allow the result of treatment to be estimated. The remaining 17 men showed a striking degree of improvement; the fissures became shallower, and in some cases the smaller fissures were no longer noticeable. In the period of our observation no tongue which was fissured lost all such marking. The improvement could be seen by the end of four weeks in 4 men; in 8 more it was evident by the end of twelve weeks, and in 2 more the beneficial effects were visible by the nineteenth week. Three of the men were not seen after twelve weeks, and it is not known whether the improvement occurred later, since their absence from the prison stopped their participation at that point. It was evident to us, therefore, that 14 of the 17 men with fissured tongues made significant improvement in not over nineteen weeks' use of 50 mg. of nicotinamide daily.

Only 6 instances of dental scalloping were seen. One of these men was not interested in taking the nicotinamide. In 2 others the margin of the tongue failed to show any appreciable changes with therapy. In the other 3 there was distinct improvement, amounting in 2 instances to disappearance of the scalloped border.

Because of variations in cooperation in one way or another of the 23 men, our data on 9 of

From the Department of Medicine of the University of Wisconsin.

1. Kruse, H. D.: The Lingual Manifestations of Aniacinosis, with Especial Consideration of the Detection of Early Changes by Biomicroscopy, Milbank Mem. Fund Quart. **20**:262, 1942.

2. Kyhos, E. D.; Gordon, E. S.; Kimble, M. S., and Sevringhaus, E. L.: The Minimum Ascarbic Acid Need of Adults, J. Nutrition **27**:271, 1944.

them do not indicate the condition of the papillae with sufficient exactness to permit conclusions to be drawn. It is certain that at the conclusion of the experimental period the papillae were in a stage of excellent development and were numerous in all the 20 men who were observed at the nineteenth week. In fact, at this observation only 1 of them could be said to have any deficiency in the number or the development of the papillae. The appearance of the tongues in 14 men had demonstrated to us the progressive changes which could not be overlooked, advancing from virtual absence of papillae in 1 and scanty papillae in the others to a normal appearance in the whole group. We are sure that the improvement was complete in 5 men after twelve weeks and that the conditions were distinctly improved in the others by the nineteenth week.

After this period of treatment, some of the men were changed to a dosage of 100 mg. of nicotinamide daily, and 4 were given riboflavin (1 mg. each day). During the next few months several observations were made, but we were not convinced of any further improvement in fissures, scalloped borders or papillae.

These observations do not constitute a final appraisal of the changes in the tongue described by Kruse, but they have convinced us of the validity of several of the criteria he has suggested for the later stages of chronic aniacinosis. Furthermore, we believe that the use of a dose of 50 mg. of nicotinamide is probably as effective as the larger dose used by Kruse, 200 mg. The completeness of recovery from the atrophy of substance evidenced in part by the fissures cannot be determined from our data. It may be possible that fissures can be eliminated in many cases. We are inclined to doubt this, suspecting that scars of the deeper fissures may prevent a complete restoration of the tongue to a normal state. It is also possible that there are other causes of fissuring in the tongue. Clinical experience with patients who were given large doses of nicotinamide for many months in order to test this hypothesis has contributed to this doubt. For example, a young man was seen because of extensive fissuring of his tongue of many years' standing, with burning paresthesias of the tongue. A previous clinical consultant had prescribed 200 mg. doses of nicotinamide daily, which had been taken for over one year without benefit. It was then suggested that he desist from smoking, whereupon the paresthesias diminished rapidly, although the fissures were unchanged.

Apparent improvement in growth of papillae on the dorsum of the tongue with nicotinamide therapy led to a trial of this treatment in several cases of severe anemia with atrophy of the dorsal papillae. Success in such cases justifies the use of this therapy in a large number of patients with pernicious anemia. Two cases may be summarized as follows:

E. D. W., a 54 year old diabetic woman, was moderately obese, suffered some symptoms of the climacteric and was hypertensive but without cardiac enlargement or renal damage. Gastric distress, fatigue and paresthesias in the legs had led the referring physician, a year before our examination, to a diagnosis of pernicious anemia and to the use of liver and iron therapy. The tongue had been fissured for about three years. It was only suggestively atrophic but was completely "bald." No free gastric acid appeared after the hypodermic administration of 1 mg. of histamine phosphate. The blood contained 15 Gm. of hemoglobin per hundred cubic centimeters, with 5,000,000 red blood cells per cubic millimeter. The diagnosis of pernicious anemia was therefore considered unproved. Treatment was based on dietary control of the diabetes and obesity, with use of diethylstilbestrol for the climacteric syndrome. Because the gastric discomfort which resulted and in spite of relief from most symptoms, other estrogenic materials were tried, and the patient was found to prefer a natural estrogenic substance.

After an interval of six months she returned with continued gastric discomfort and soreness of the tongue, which was unusually red in the midline and increasingly atrophic. The blood picture had changed in the previous five weeks from 10.3 to 8 Gm. of hemoglobin, 3,170,000 to 1,970,000 red blood cells and 6,750 to 3,400 white blood cells. The mean corpuscular volume was 129 cu. mm. and the saturations index 0.93. She was given 15 unit doses of liver extract U. S. P. daily, and on the fifth day the reticulocytes had risen from 1 to 18.2 per cent. The treatment was continued at 15 units weekly after her discharge. When reexamined three months later the blood contained 14.3 Gm. hemoglobin and 5,310,000 red blood cells. The diagnosis of pernicious anemia appeared adequately confirmed.

Coincident with the resumption of the liver therapy, this patient was started on 100 mg. doses of nicotinamide daily. Through an error of the pharmacist, the prescription was filled with nicotinic acid, which the patient took for six months in spite of the vasomotor disturbances before the error was detected from her report and the correct drug put into use. But by the end of three months' use of nicotinic acid, the papillae had become more numerous and the fissures shallower, and the burning of the tongue was no longer noted. By the end of one year the tongue looked essentially normal, as to both fissures and papillae. It had been established that 15 units of liver every ten days was an adequate dose but that if the interval was three weeks a relapse occurred. During this relapse, the tongue did not show regression. It appears, therefore, that atrophy of the tongue as seen in pernicious anemia is not reversible by the application of adequate doses of liver but may be reversed by nicotinic acid or nicotinamide. It is equally evident that the nicotinamide therapy did not prevent a relapse of the anemia. Observations in another case of diabetes with pernicious anemia substantiated these conclusions.

A third case was that of E. W. L., a 62 year old farmer with mild diabetes and generalized arteriosclerosis, who complained of epigastric distress after meals, constipation, sore tongue the previous year with relief after use of some preparation of vitamin B complex and weakness, paresthesias and clumsiness in the legs.

The tongue was atrophic and essentially devoid of papillae and had numerous fissures. Vibratory sense in the legs was greatly impaired; tendon reflexes were reduced, and motor tests showed ataxia. Roentgenographic study revealed only a small duodenal diverticulum. There was no production of free gastric acid after injection of 1 mg. of histamine phosphate. The blood contained 11.5 Gm. of hemoglobin, 3,140,000 red blood cells and 5,350 white blood cells. The mean corpuscular volume was 118 cu. mm. and the saturation index 0.98. During the course of eight days he was given 50 units of crude liver extract, but over the two weeks' period of observation there was no detectable rise in reticulocytes above the 0.4 per cent level. No noticeable change in red blood cells was observed. During the same interval he was given 150 mg. of nicotinamide daily, which led to noticeable development of papillae on the dorsum of the tongue within the first week and further improvement in the second week, and the treatment was continued after his discharge. A routine was established with use of ferrous sulfate and hydrochloric acid for the anemia, since it was felt that the diagnosis of pernicious anemia could not be maintained in the face of a negative response to potent liver.

When he was reexamined after one month, the blood picture was somewhat improved (12.5 Gm. hemoglobin and 3,408,750 red blood cells). The complaints were unchanged, but the papillae on the tongue were still well developed. Examination of the spinal fluid gave no evidence of syphilis or any other lesion of the spinal cord. It was concluded that this was a case of hyperchromic anemia with combined systemic disease, possibly due to prolonged multiple nutritional deficiencies. Therefore, liberal use of protein supplemented by a crude liver extract orally was continued. Further observations have been precluded by his failure to return to the clinic. It seems fair to conclude that the nicotinamide therapy was effective in producing regeneration of papillae on the tongue in this atypical anemia, in spite of incomplete remission in the anemia.

SUMMARY

Observations on an unselected group of prisoners showed that nearly one third had visible evidence of atrophy in the tongue, including fissures, reduced number of filiform papillae and dental scalloping. Treatment with 50 mg. doses of nicotinamide brought about striking improvement, most complete in the case of the papillae but also noticeable in the shallower fissures and decreased amount of scalloping. The application of similar therapy to 3 patients with fissures and atrophy of papillae, 2 with pernicious anemia and 1 with a similar anemia but without reticulocyte response to liver was followed by prompt improvement likewise. The improvement with therapy requires several months to become complete. It is concluded that the criteria of chronic aniacinosis proposed by Kruse are valid.

The expenses of this investigation have been met in part by a special grant from the Nutrition Foundation to one of us (E. L. S.).

Mr. I. C. Breitlow, of the hospital staff at the Wisconsin State Prison, and Warden L. F. Murphy cooperated to make these observations possible. Dr. J. M. Carlisle, of the E. R. Merck Laboratory, supplied the nicotinamide and riboflavin used.

ARTERIOSCLEROTIC PERIPHERAL VASCULAR DISEASE IN DIABETES

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Two recent trends in the field of diabetes make it of special interest to study the relation of diabetes to arteriosclerosis. One is the increased prominence of arteriosclerosis as a cause of death among diabetic persons. According to Joslin,[1] deaths from this cause have risen threefold, while deaths from diabetic coma have dropped to one sixteenth of their former incidence. This circumstance may in part be due to the fact that many diabetic persons are now surviving long enough to experience advanced arterial changes. The second trend is the tendency of the physician to pay less attention to hyperglycemia and glycosuria in insulin-controlled cases. A higher incidence of premature arteriosclerosis under the newer regimens of liberal diets and insulin might indicate the need for a return to the previously accepted, more rigid standards of control.

It is well known that arteriosclerosis occurs with high frequency among diabetic persons. Some believe this frequency is due to the abnormal metabolic relationships found in diabetes. Other factors suggested are inadequacy of control of glycosuria, severity of the disease, obesity and the presence of coexisting hypertension. This report summarizes our observations with respect to the relations between these factors and the incidence of arteriosclerosis as manifested in peripheral vascular disease of the lower extremities.

SCOPE OF THE WORK

Observations were made on 249 male patients attending the diabetic clinic. Data were obtained with regard to age, duration of diabetes, severity of the disease, use of insulin, adequacy of control, presence of obesity and arterial tension. The patients were carefully questioned as to the presence of intermittent claudication. The peripheral pulses of the lower extremities, including those of the femoral, popliteal, anterior and posterior tibial and dorsalis pedis vessels, were examined by palpation. When the dorsalis pedis artery alone could not be felt, the case was not considered an instance of peripheral vascular disease. Absence of dorsalis pedis pulsation can be noted in a small percentage of normal persons, while in some instances this vessel has an atypical location. Oscillometric readings were made in about half the cases. In general, the instrument readings confirmed the observations made by palpation. Other workers have found that this method of determining the presence of peripheral vascular disease, namely by palpation of the vessels, corresponds well with other tests used for appraising the arterial circulation in the extremities.[2] Since arteriosclerosis may occur occasionally without signs or clinical symptoms, it is likely that in this survey some instances of arteriosclerosis have been missed.

INCIDENCE OF PERIPHERAL VASCULAR DISEASE

In Relation to Age.—Figures for age distribution and incidence of peripheral disease appear in table 1. Of the group as a whole, 51 per cent had peripheral vascular disease.

While the number of young diabetic persons is small, there being but 19 patients under 40 years of age, 5, or approximately one fourth, had clinically apparent peripheral vascular disease. In the 41 to 50 year age group, one third of the patients had some peripheral vascular involvement. In the age group of 51 to 60 years 42 per cent had some peripheral vascular disease. Beyond this age group there were 125 patients, of whom 63 per cent had evidence of peripheral vascular abnormality. As might be anticipated, the highest incidence was found in the oldest age group.

In Relation to Duration of Diabetes.—In table 2 are summarized the data relating to the

From the Metabolism Clinic and the Peripheral Vascular Disease Clinic, Mount Sinai Hospital.

1. Joslin, E. P.: Treatment of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1940.

2. (*a*) Kramer, L. I.: Various Methods of Determining the Early Diagnosis of Arteriosclerosis in Diabetes, New England J. Med. **220**:278 (Feb. 16) 1939. (*b*) Homans, J.: Circulatory Deficiency in Relation to Medico-Legal Problems, Ann. Int. Med. **18**:518 (April) 1943.

duration of diabetes. Of patients with known diabetes for less than three years, 41 per cent had peripheral vascular disease. Among a total of 94 patients whose recognized diabetes was of less than five years' duration, 39, or 42 per cent, showed evidence of peripheral vascular disease. There were 154 patients with diabetes of more than six years' duration. In this group there were 89 instances of peripheral vascular disease, a percentage of 58. The table indicates the high incidence of peripheral vascular disease even among those patients in whom the diabetes is not long established.

Observations similar to ours are reported by other workers. In a roentgenologic study of the extremities of diabetic patients, Bowen, Koenig and Viele [3] found evident arteriosclerosis in the vessels of the legs in 50 per cent of their patients. In reports by Morrison and Bogan,[4] Shepardson,[5] Kramer,[2a] Kramer,[6] Dry and Hines,[7] Meyers and Altschuler [8] and Eisele,[9] the incidence of peripheral vascular disease in diabetic persons

TABLE 1.—*Peripheral Vascular Disease in Various Age Groups*

Age Group	Total No. of Patients	P. V. D. Absent		P. V. D. Present	
Under 30 years	9	7	74%	2	26%
31 to 40	10	7		3	
41 to 50	32	21	(66%)	11	(34%)
51 to 60	73	42	(58%)	31	(42%)
Over 61	125	46	(37%)	79	(63%)
Total	249	123	(49%)	126	(51%)

ranged from 17 per cent to as high as 68 per cent. Hines and Barker [10] found that among 280 general patients with arteriosclerosis obliterans one fifth had diabetes. Wilder,[11] reporting on observations made at necropsies of diabetic patients under 40, found that arterial occlusion sufficient to cause symptoms was present in 39 per cent of the cases.

In Relation to Severity of Diabetes.—Our patients were classified into three groups according to the severity of the disease: mild, moderately severe and severe diabetes. In the group with mild diabetes were those whose blood sugar levels were not appreciably elevated and who required no insulin or required only amounts up to 15 units. Most required no insulin. The patients in the group with moderately severe diabetes required from 15 to 45 units. Patients who required amounts of insulin larger than this were considered to have severe diabetes.

TABLE 2.—*Peripheral Vascular Disease in Relation to Duration of Diabetes*

Years' Duration	Total No. of Patients	P. V. D. Absent	P. V. D. Present
Under 3	66	39 (59%)	27 (41%)
4 to 5	28	16 (57%)	12 (43%)
6 to 10	70	29 (41%)	41 (59%)
11 or more	84	36 (43%)	48 (57%)

The data given in table 3 indicate no relation between the incidence of peripheral vascular disease and the severity of the diabetes. Among those with mild and moderately severe diabetes the incidence of peripheral vascular disease was equally distributed. When those with moderately severe and those with severe diabetes were grouped together, there were 54 instances of peripheral vascular disease, or 49 per cent, as compared with 54 per cent for those with mild diabetes. Other authors [12] also found no relation between the severity of diabetes and the extent of peripheral vascular disease.

TABLE 3.—*Severity of Diabetes and Peripheral Vascular Disease*

Severity of Disease	Total No. of Patients	P. V. D. Absent	P. V. D. Present
Mild	137	63 (46%)	74 (54%)
Moderately severe	82	37 (45%)	45 (55%)
Severe	29	20 (69%)	9 (31%)

In Relation to Degree of Diabetic Control.—Persistent glycosuria with significant elevation of the blood sugar was considered poor control; freedom from glycosuria was considered good control, while fair control included intermittent glycosuria.

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11. Wilder, R. M.: Diabetic Arteriosclerosis, Internat. Clin. **2**:13 (June) 1939.

12. Shepardson.[5] Meyers and Altschuler.[8]

Among the 155 patients with well controlled diabetes (table 4), 78, or about one half, had peripheral vascular disease. In the less satisfactorily controlled group, or "fair," 29 out of 48 patients, or 60 per cent, had peripheral vascular disease. In the poorly controlled group, an increased incidence of occluded vessels was not found. In this group the rate was 33 per cent. The low rate among the patients with poorly controlled diabetes was not due to the fact that this group was predominantly young.

Some affirm that with better control of diabetes less arteriosclerosis is evident. White [13] reported the observation of arteriosclerosis, as demonstrated by roentgenographic or ophthalmologic examination or by autopsy, in 52 young diabetic persons with onset of diabetes in childhood. No sclerosis was observed when the blood sugar and blood cholesterol levels were below 200 mg. Sclerosis increased with elevation of blood cholesterol levels. In connection with these abnormal changes, hypercholesteremia appeared more significant than hyperglycemia. In the opinion of Bowen, Regan and Koenig [14] good control of diabetes was an influential factor in preventing and delaying arteriosclerosis in the lower extremities.

TABLE 4.—*Control of Diabetes and Peripheral Vascular Disease*

	Total No. of Patients	P. V. D. Absent	P. V. D. Present
Good	155	77 (50%)	78 (50%)
Fair	48	19 (40%)	29 (60%)
Poor	42	28 (67%)	14 (33%)

TABLE 5.—*Obesity and Peripheral Vascular Disease*

Type	Total No. of Patients	P. V. D. Absent	P. V. D. Present
Never obese	87	40 (46%)	47 (54%)
Obese at time of examination or previously	160	79 (50%)	81 (50%)

TABLE 6.—*Hypertension and Peripheral Vascular Disease*

Group	Total No. of Patients	P. V. D. Absent	P. V. D. Present
No hypertension	138	71 (51%)	67 (49%)
Hypertension present	99	44 (44%)	55 (56%)

In Relation to Obesity.—The factor of obesity in our patients was gaged by an appraisal of present and past weights. Some of the men were thin and of asthenic habitus and had never weighed more than 150 pounds (68 Kg.). Others were obviously of heavy build and weighed more than this either previously or at the time of examination.

The incidence of peripheral vascular disease was about equally distributed as far as obesity was concerned. It occurred just as often in the nonobese as it did in the obese patients (table 5).

In Relation to Hypertension.—Patients whose systolic tension was 150 mm. of mercury or over with a diastolic pressure of 90 mm. or above were classified in the hypertensive group. Those with blood pressures below these levels were regarded as nonhypertensive. The figures are given in table 6.

Sixty-seven of the nonhypertensive patients, or 49 per cent, had peripheral vascular disease, while 55 of the hypertensive group, or 56 per cent, had arterial involvement. The difference is insignificant.

COMMENT

Type and Sites of Arteriosclerotic Lesions.—The foregoing tables indicate the high incidence of peripheral vascular disease among the diabetic patients we examined. The incidence of the condition appeared to be unrelated to such factors as duration of the diabetes, severity of the disease, effectiveness of control and existence of obesity and of hypertension.

The early and apparently selective appearance of arteriosclerosis in diabetes is not limited to the vessels of the extremities. In this disorder, arteriosclerotic lesions involve other organs as well; hence peripheral sclerosis is only part of a more general picture.

Localization of arteriosclerosis in the heart and aorta is exceedingly common. This has been established on the basis of necropsies by numerous investigators, including Joslin,[15] Root,[16] Warren,[17] Nathan [18] and Root and Sharkey.[19] Joslin declared the heart to be the most frequent

13. White, P.: Diabetes in Childhood, in Joslin, E. P.: Treatment of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1935.

14. Bowen, B. D.; Regan, J. S., and Koenig, E. C.: The Development of Arteriosclerosis in the Diabetic, Ann. Int. Med. **12**:1996 (June) 1939.

15. Joslin, E. P.: Arteriosclerosis in Diabetes, Ann. Int. Med. **4**:54 (July) 1930.

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17. Warren, S.: Pathology of Diabetes, Philadelphia, Lea & Febiger, 1938.

18. Nathan, M. H.: Coronary Disease in One Hundred Autopsied Diabetics, Am. J. M. Sc. **183**:495 (April) 1932.

19. Root, H. F., and Sharkey, T. P.: Arteriosclerosis and Hypertension in Diabetes, Ann. Int. Med. **9**:873 (Jan.) 1936.

site of arteriosclerosis in persons with diabetes. Root and Sharkey stated that coronary disease as a cause of death is four times as frequent in diabetic persons as in nondiabetic, while Nathan found coronary disease six and a half times as high.

The earlier appearance of arteriosclerosis in diabetic persons as compared with its appearance in normal persons is also recognized. Warren, as cited by Joslin,[15] Ophüls,[20] Lisa, Magiday, Galloway and Hart [21] and Lehnherr [22] have presented reports of necropsies in support of this fact.

From the standpoint of pathologic characteristics, the actual lesion found in diabetic persons does not differ from that in nondiabetic. This lack of difference has been pointed out by Ophüls,[20] Hines and Barker,[10] Lisa and others,[21] Sappington and Fischer [23] and Lisa, Magiday and Hart.[24] The lesions consist essentially of intimal thickening, subintimal deposition of fatty material, including cholesterol, and degenerative changes in the media with a tendency to ultimate thrombosis of the vessels.

Abnormal Metabolism and Arteriosclerosis.—There is no uniformity of opinion as to what initiates these arteriosclerotic changes in diabetic patients. The occurrence of marked elevation of the blood lipids in diabetes, as cited by Rabinowitch,[25] was long ago pointed out by Joslin, Bloor and Gray. Consequently, the hypercholesteremia of diabetic patients has most frequently been held responsible for the early appearance of arteriosclerosis. Emphasis on blood lipids, especially cholesterol, as specific metabolites of first importance also arose from the belief of Virchow and Aschoff that the intimal changes in arteriosclerosis represented the aging of arterial tissues through stress and strain and that fat from the blood stream was imbibed and deposited in the intima. Anitschkow [26] experimentally produced in rabbits the typical picture of atherosclerosis by feeding them pure cholesterol. Similar experiments were extensively reported on by Leary,[27] who also concluded that the lesions of atherosclerosis were due to hypercholesteremia. Other workers, including Bowen, Regan and Koenig,[14] Hines and Barker,[10] Rosenthal [28] and Rosenblum,[29] likewise concluded that an altered cholesterol metabolism was related to the development of arteriosclerosis.

Agreement on this point does not exist. Weiss and Minot [30] regarded as equivocal the evidence presented to demonstrate a reciprocal relation between the cholesterol content of the diet and the development of arteriosclerosis. Duff [31] took issue with the experimental work of Leary [27] and cited the occurrence of arteriosclerosis in man without deviation of the blood cholesterol from normal limits. Hirsch and Weinhaus [32] also called attention to the development of arteriosclerosis in adult life in the absence of an appreciable elevation of blood cholesterol or lipids. As related to the process in diabetes, Hunt [33] found lowest cholesterol values in persons with greatest arteriosclerosis. Among 6 juvenile diabetic patients under 20 years of age, all of whom had roentgenologic evidence of arteriosclerosis, 4 had normal cholesterol values.

Winternitz expressed the opinion that the earliest changes leading to atheromatous plaque formation were due to small thrombi and hemorrhages arising in the tiny vasa vasorum of the intimal walls of vessels in which the elasticity and contractility were diminished. Blumenthal, Lansing and Wheeler [34] have recently presented

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22. Lehnherr, E. R.: Arteriosclerosis and Diabetes Mellitus, New England J. Med. **208**:1307 (June 22) 1933.

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29. Rosenblum, M. H.: A Consideration of Disease of the Blood Vessels in Diabetes Mellitus, Ohio State M. J. **38**:46 (Jan.) 1942.

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their own material supporting the concept of numerous other workers as well that changes consisting of demonstrable calcification first occur in the medial wall and that the intimal changes with lipid deposition are secondary. Should these theories concerning the genesis and development of arteriosclerosis be correct, it is not apparent why the process should occur more prominently in diabetic persons.

Warren [17] stated that fluctuations in the blood sugar concentration might produce osmotic changes affecting the permeability of the intimal ground substance, thereby opening it up to deposition of lipids. Wilder and Wilbur [35] expressed the opinion that hyperglycemia, ketosis, frequent infections, periodic dehydration or inadequacies of the diet in certain nutritional factors might be contributory causative factors.

Moschcowitz [36] suggested that arteriosclerosis, differentiating it from atherosclerosis, found in diabetic patients may itself be responsible for the diabetic condition. His concept is that an arteriocapillary fibrosis in the islands of Langerhans produces the insufficiency which results in diabetes. There are few adherents to this concept, according to Lukens,[37] who, in discussing the pathogenesis of diabetes, stated that advanced pancreatic arteriosclerosis can exist without the presence of diabetes.

Finally, Dry and Hines [7] postulated a biologic background to explain the advanced arteriosclerosis in diabetic patients. They expressed a belief in an inherent weakness affecting the insulin-producing tissues and the vascular system which requires only an additional stimulus to bring out the pathologic changes in the tissues. In the case of the insulin apparatus, the stimulus can be infectious illness, overfeeding or obesity. In the case of the vascular system, the stimulus can be hypertension, obesity or lipemia.

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SUMMARY

Of 249 male diabetic patients evidence of peripheral vascular disease was present in 51 per cent. It was found in one fourth of the patients under 40 years of age. While it was found in greater proportion in patients whose diabetes was of long standing, as many as two fifths of those with less than three years' history had some peripheral vascular disease. It occurred with equal frequency among the patients with mild diabetes and among those with more severe diabetes who used insulin. Control of the disease, reflected by minimal glycosuria, did not coincide with a lower incidence of peripheral vascular disease. Among the lean men the condition was found in the same proportion as among the obese. The slightly lower incidence in the nonhypertensive patients, 49 per cent as against 56 per cent, did not seem significant. The only apparent common denominator was the diabetic condition itself.

CONCLUSIONS

Confirming the work of others, our observations indicate that an apparently selective and frequently premature arteriosclerosis occurs in the peripheral vessels of the lower extremities in diabetic patients.

The increased incidence of arteriosclerosis in diabetic patients, irrespective of the usually accepted factors which determine arteriosclerosis in nondiabetic persons, may indicate a metabolic origin or imply an acceleration of the process by some metabolic factor. From the statistical standpoint of our study, the relationship between such factors and peripheral vascular disease is not made evident.

The presence of cholesterol deposits in the arteriosclerotic lesion and the hypercholesterolemia of patients with neglected or poorly controlled diabetes suggest a linkage between the two disorders. However, no such connection has so far been unequivocally established. It does not of course follow that in the treatment of diabetes such factors as control of weight and hypertension can be ignored or that suitable dietary and insulin regimens can be neglected.

EFFECT OF INTRAVENOUSLY ADMINISTERED SOLUTION OF ACACIA ON ANIMALS

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Considerable attention has been focused on intravenous administration of solution of acacia, the probable physiologic and physical action of acacia so administered and its fate in the organism. The study herein reported was a long term investigation of dogs which had received intravenously large doses of a solution of acacia similar to that used in treatment of patients who display the resistant edema that may accompany glomerulonephritis and the nephrotic syndrome. The purpose was to note the general effect on the animals as well as the specific effect on the liver.

MATERIAL AND METHODS

Three normal adult dogs, which were receiving a diet of ground raw meat, cracker meal and milk in daily amounts calculated to maintain their body weight, were used in this study. Beginning on Jan. 7, 1942, each dog received intravenous injections of 6 per cent acacia in 0.06 per cent isotonic solution of sodium chloride, as is indicated in table 1. The solution was warmed to approximate body temperature, and reactions were observed after the injections. Additional amounts of acacia were injected each two or three days in order to maintain the acacia in the serum of the 3 dogs at 1.5, 3 and 3.5 per cent, respectively. Injections of acacia were discontinued after seventy-six days, on March 23.

The Experimental Dose of Acacia Compared with the Therapeutic Dose.—In table 1 are recorded the total quantities of acacia injected into each animal. The average patient with chronic glomerulonephritis and the nephrotic syndrome who receives therapeutic injections of solution of acacia for resistant edema is given from 90 to 120 Gm. of acacia, or from 1.3 to 1.8 Gm. per kilogram of body weight. The doses given the animals, as recorded in table 1, represent from eight to thirty-seven times as many grams of acacia as is received by the average patient. The amounts administered to the animals are equivalent to doses of 1,068 to 3,244 Gm. of acacia given to an average patient who weighs 68 Kg. Of patients with chronic glomerulonephritis and the nephrotic syndrome treated at the Mayo Clinic, the one who received the largest quantity of solution of acacia during the period of hospitalization was given 330 Gm., or 4.9 Gm. per kilogram of body weight. This dose is much greater than the average total dose of 90 Gm. divided into three injections of 30 Gm. each, which is usually administered to patients. Of the 3 dogs, the 1 which received the least acacia, and in the serum of which the concentration of acacia was lowest, was given slightly more than three times the number of grams per kilogram of body weight that was given to the patient who received the most. The dog which was given the most acacia per kilogram of body weight, and the concentration of acacia in the serum of which was maintained at the highest level, received nine and seven-tenths times the number of grams that the patient just mentioned received. This patient was living, felt well and was working daily when this paper was written. The dogs received approximately three times the number of injections of acacia given to this patient.

TABLE 1.—*Acacia Intravenously Administered and the Concentration of Acacia in the Blood Serum Throughout the Period of Injection*

Dog		Injection Period, Days	Total Injections	Total Acacia Injected, Gm.	Acacia Injected per Kg. Body Weight, Gm.	Acacia in Serum, Average Gm. per 100 Cc.
Number	Weight, Kg.					
9	6.4	76	32	100.6	15.7	1.4
7	7.0	76	35	277.8	39.7	3.0
8	5.8	76	35	276.6	47.7	3.4

Biopsy.—With the help of Dr. F. C. Mann, abdominal exploratory operations and biopsies of the livers were performed at four different periods following discontinuance of the injections of acacia: first, at the time of completion of the administration; second, ten weeks later; third, approximately thirteen months, and, fourth, twenty-six months, after the discontinuance of the injections of acacia. Specimens for biopsy were taken from the spleens of 2 animals at the time of the last operations. At the time of these exploratory operations, the organs were inspected and specimens of tissue were obtained for chemical examination. Microscopic examination of the removed specimens was made by Dr. Baggenstoss.

Technics Employed.—The concentration of acacia in the blood serum and in the urine was determined by

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precipitation of trichloroacetic acid filtrates with acetone, according to the principles of the method described by Power.[1] Weighed portions of tissues and stools were macerated in freshly prepared isotonic solution of sodium chloride and made up to definite volumes with trichloroacetic acid. Acacia was determined by precipitation of aliquot portions of the filtrate. Glycogen, if present in normal amounts, may be expected to interfere with an exact determination of acacia by this method. Consequently, approximate colorimetric determination of acacia present in the tissue filtrates was accomplished by application of Bial's orcin test for pentose.

Blood for analysis was obtained from the veins of the legs and by puncture of the jugular vein. When plasma was used, potassium oxalate, 5 mg. per cubic centimeter, was used as an anticoagulant. Determinations of protein fractions in the blood serum were made in duplicate by Howe's method as described by Peters and Van Slyke, using 22.5 per cent sodium sulfate at 37 C. Fibrinogen was determined by the following method: Five-tenths cubic centimeter of oxalated plasma was mixed with 24 cc. of 0.9 per cent sodium chloride solution and 0.5 cc. of 2.5 per cent calcium chloride, and the mixture was allowed to stand overnight. The fibrin clot was removed by filtration, and 20 cc. of the filtrate was analyzed for nitrogen by a modified Kjeldahl procedure. For determinations of hemoglobin, the Cenco-Sheard-Sanford photelometer was used. The quantity of cells per hundred cubic centimeters was determined by centrifuging in hematocrit tubes. Mean erythrocyte determinations were made by means of the Haden-Hausser erythrocytometer.[2] The sulfobromophthalein sodium test was performed by Rosenthal's method.[3] For determination of coagulation time, the Lee and White method was employed.[4] The prothrombin time was determined by use of Quick's method.[5]

EXPERIMENTAL RESULTS COMPARED WITH THERAPEUTIC RESULTS

At the outset of this section of the paper, it may be advisable to explain how well, from the standpoint of tenure of life, the dogs withstood the combined experimental procedures. About three months after discontinuance of administration of acacia and after the 3 animals had recovered from two exploratory operations, 1 dog began to eat poorly, and his weight gradually decreased. He died four months after the last injection of acacia. Postmortem examination did not reveal any specific cause of death; the liver was enlarged, but the other organs appeared normal. The other 2 animals recovered normally from four exploratory operations and were still living at the time of writing of this paper.

Concentration of Acacia in the Blood Serum.—The average value at which the concentration of acacia was maintained throughout the period of injection in the serum of the 3 animals is given in table 1. After discontinuance of the injections, the concentration of acacia in the serum of the animals immediately began to decrease, as is

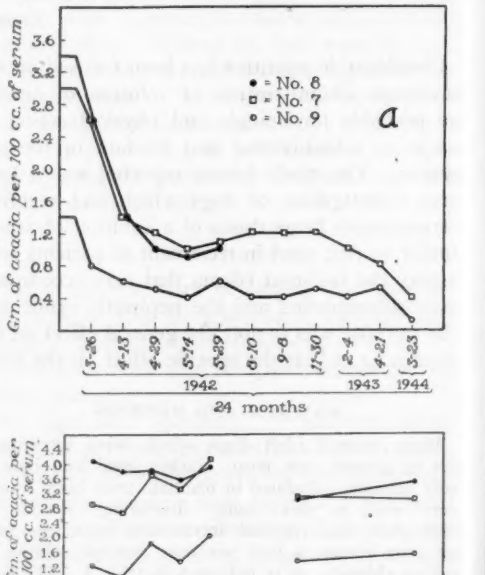

Fig. 1.—(*a*) Rate of disappearance of acacia from the blood stream. Solution of acacia was injected intravenously through a period of seventy-six days, as it was necessary to keep the concentration of acacia in the serum constant. Then injection was discontinued. The value at the extreme left of each curve represents the value at the time when injection was stopped. Values to the right were determined in the subsequent two years. (*b*) Concentration of acacia in the serum and quantity in the urine. Through two portions of a total period of seventy-six days, solution of acacia was injected intravenously and the indicated determinations were made. The curves at the left represent values of the first portion of the period and the curves at the right, values of the second portion. Injections were as follows: Animal 7: 21 Gm. of acacia given on January 7, 9 and 11; 9 Gm. on March 16. Animal 8: 34.8 Gm. of acacia given on January 7 and 9; 9 Gm. on March 16. Animal 9: 6.3 Gm. of acacia given on January 7, 9 and 11; 3 Gm. on March 16.

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shown in figure 1 *a*. Two years after discontinuance of the injections of acacia, it was noted that some acacia still was present in the circulating blood, although the concentration was much reduced from that which had obtained formerly.

In the average clinical case in which the intake of fluid is controlled and in which the total dose of acacia is the average one of 90 Gm., the immediate concentration is approximately 2 Gm. per hundred cubic centimeters of blood serum. When patients receive this average dose in treatment of the resistant edema of chronic glomerulonephritis and the nephrotic syndrome and are examined one year after the end of the treatment, one finds that the average concentration of acacia in the serum is approximately 0.1 Gm. per hundred cubic centimeters. The concentration of acacia in the blood serum of 1 patient, who recently returned for reexamination six years after her last injection of acacia, was 0.01 Gm. per hundred cubic centimeters. She had received the average total dose of 90 Gm. of acacia.

Excretion of Acacia in Urine and Stool.—Acacia is not readily metabolized by the animal body and is in part excreted in the urine in a state which has not become so modified as to lose its property of precipitating in the presence of acetone.

The greatest excretion of acacia in the urine of the 3 animals which are the subjects of table 1 and figure 1 occurred in the periods immediately following injections of acacia (fig. 1 *b*). In two trial periods, the animals which were given more solution of acacia excreted more. The quantity of acacia excreted is small compared to the amount administered or compared to the concentration of acacia in the serum. The amount of acacia excreted is not proportional to the amount of urine collected in twenty-four hours, for the largest amount of urine sometimes contained the least acacia. Two years following cessation of injections of acacia, traces of acacia still were being excreted in the urine of the 2 living animals.

Certain authors, including Huffman,[6] have reported considerable variation in the amount of acacia which could be recovered from the urine of individual patients. In some cases Huffman was able to recover more than half of the total amount injected intravenously. Hartmann[7] found that acacia appeared in the urine immediately after its intravenous injection and that the rate of excretion varied with different persons. He also stated that the average excretion was about 25 per cent in the first two days and 60 per cent during the first seven. Moreover, he noticed a maximal concentration of acacia in the urine of 1.3 per cent. Andersch and Gibson[8] found that only small amounts of acacia were eliminated in the urine of animals. They reported that only 20 per cent of the total acacia which was injected intravenously into a patient with nephrosis was recovered in the urine over a period of six weeks; three of the six weeks over which urine was collected followed the last injection. They also reported that the greatest excretion occurred during the period in which the acacia was administered. Butt, Power and Keys[9] reported that about 20 per cent of the total quantity of acacia administered in single or multiple intravenous injections was excreted in the urine in the first eight days after injection and that most of the acacia excreted appeared in the first three days after its administration. Acacia was present in the stool, but in questionable amounts, as the acetone precipitate in normal stool was not determined. Determinations were made at the time the injections of acacia were discontinued.

Effect on Structure of Liver, Spleen and Kidney.—On gross examination of each of the 3 animals at the time of the first exploration, the day after the last injection of acacia, the liver was estimated to be increased from one fourth to twice normal size. The liver which was most enlarged was that of the animal which received the most acacia, and this liver appeared lighter in color than the livers of the other 2 animals and than the normal livers. The livers of all 3 animals had smooth surfaces and rounded edges, in the latter respect resembling the appearance of a restored liver. They were firmer than normal livers on palpation. Little bleeding occurred from the cut surfaces of the livers of the 2 animals which received the most acacia; the third bled normally. In the abdominal cavity of the animal which had received the most acacia was several hundred cubic centimeters of clear ascitic fluid.

The animals recovered normally from the first exploration. Conditions at the time of the second examination, ten weeks later, were little

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changed from those at the time of the first. The livers continued to be enlarged. The surfaces were smooth, and the edges were rounded and not friable. The livers were slightly firmer than normal. They had undergone repair after excision of tissue, as would normal livers. No ascites, however, was found. At the time of the last examination of the 2 living animals, the liver of that 1 of the 2 which had received the more acacia was still slightly, but definitely, enlarged. The edges were still rounded. The liver still was firmer than normal. The liver of that animal of the 2 which had received the less acacia appeared normal.

For comparison, a section was taken for microscopic examination from the liver of a normal animal (fig. 2 *a*). Sections of liver taken from the 3 observed animals in the first three months after discontinuance of the injections of acacia were examined microscopically. Some of the parenchymal cells in the inner zone of the lobule, about the central vein, and in the periportal areas stained more lightly and were clearer than cells of the outer, and especially the midzonal, region of the lobule. The involvement about the central vein resembled the microscopic picture seen in cases of chronic passive congestion of the liver. The central veins were compressed, and the bile ducts were partly obliterated. The parenchymal cells appeared distended, and many of the nuclei were eccentric (figs. 2 *b* and *c*). The cytoplasm of the parenchymal cells was vacuolated, so that it had the appearance of hydropic change or of a foam cell, and the picture resembled that of cells seen in renal tubules after intravenous injection of sucrose. Hepatic cells were not destroyed, although some polymorphonuclear cells were scattered through the fields.

In the specimen of liver obtained for biopsy after the fourth exploration, the parenchymal cells of the hepatic cords in the midzone of the lobule took the stain about normally, and this zone was distinct (fig. 3). It is not possible quantitatively to compare the sections studied at the time that the injections of acacia were discontinued with those obtained two years later, but there continued to be much vacuolation of the parenchymal cells throughout the sections.

Attempts were made to stain these cytoplasmic vacuoles. The vacuoles did not stain for glycogen or for fat. The mucicarmine stain gave a negative result for mucin as did also the congo red stain and the methyl violet stain of frozen sections for amyloid. These vacuolated cells may represent stored acacia, but a histologic stain positive for acacia is not available.

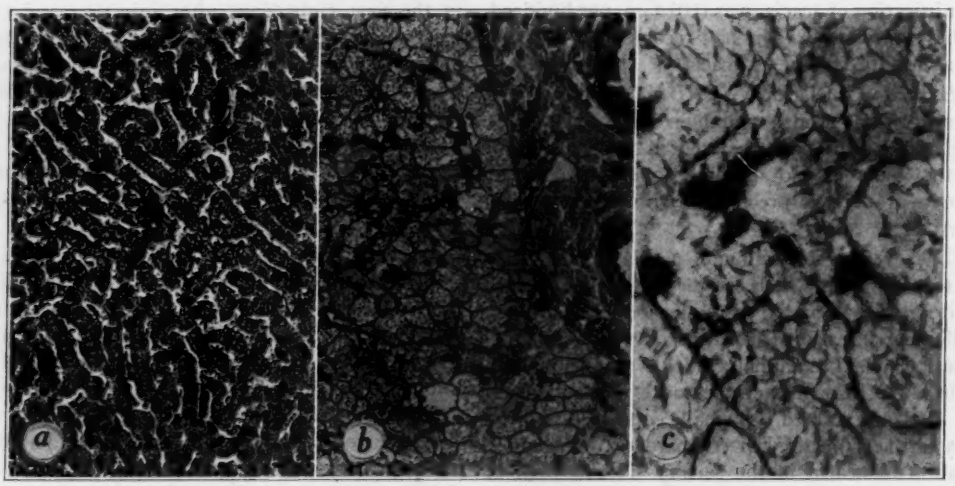

Fig 2.—Sections. (*a*) Normal liver of dog (× 160); (*b*) liver of dog when injections of acacia had just been completed (× 190); (*c*) same as *b* under higher magnification (× 960).

The capsule of the spleen and the splenic reticulum also contained relatively large and clearly staining cells, throughout the cytoplasm of which vacuoles were found (fig. 4 *a*). The involvement was less pronounced here than in the liver.

The epithelium of both the convoluted and the collecting tubules (fig. 4 *b*) of the kidney also contained vacuolated cells. The process was not as diffuse as it was in the spleen and the liver. Vacuolated cells were not seen in the glomerular tufts.

As has been noted earlier, acacia is used in the treatment of chronic glomerulonephritis with re-

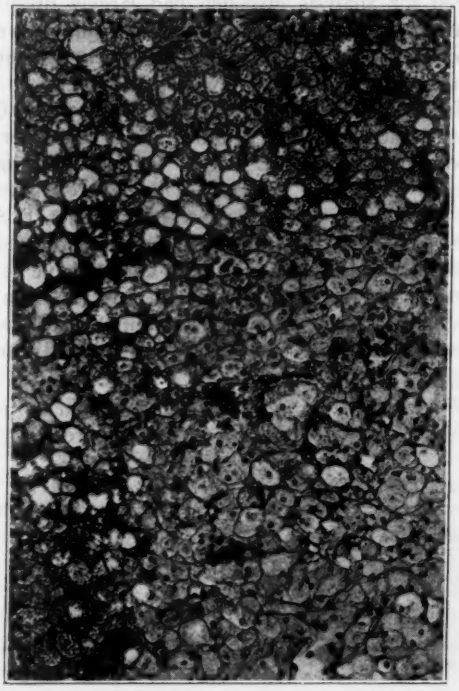

Fig. 3.—Section of liver of dog two years after injections of acacia had been completed (× 160).

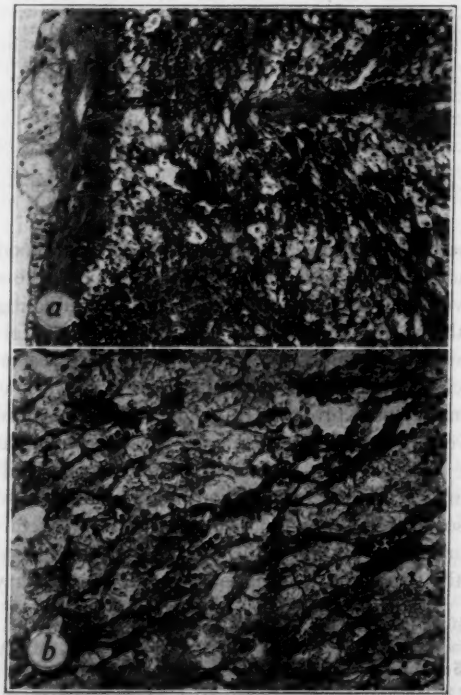

Fig. 4.—Sections. (*a*) Spleen showing vacuolation in capsule and reticulum (× 120); (*b*) convoluted tubules of kidney showing vacuolation (× 155).

sistant nephrotic edema. Also, it is used in some cases in treatment for acute shock. Hepatic, splenic and renal tissues obtained at necropsy in certain cases of these kinds were examined grossly and microscopically for the presence of the cytoplasmic vacuolation seen in the animal tissues.

In order to establish the significance of the results of examination of tissue of these patients, which presently will be described, it is necessary to record the quantity of acacia administered to them or the concentration of acacia in their blood serums or both at certain periods before their deaths. A woman, 1 of 2 patients with chronic glomerulonephritis and nephrotic edema who were treated at the clinic, had received 210 Gm. of 6 per cent solution of acacia, which amounted to approximately 3.7 Gm. per kilogram of body weight, and the concentration of acacia in the serum was 1.7 Gm. per hundred cubic centimeters three years before her death. The second patient, a man, whose diagnosis was similar, had received 183 Gm. of solution of acacia, or approximately 2 Gm. per kilogram of body weight; the concentration of acacia in the serum was 2.7 Gm. per hundred cubic centimeters three

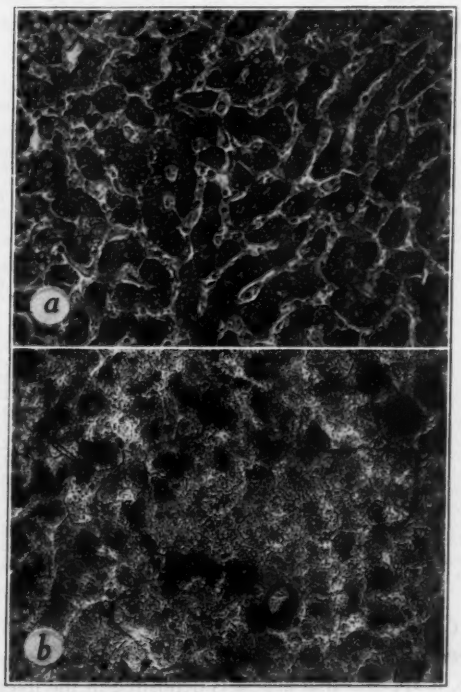

Fig. 5.—(*a*) Section of liver of man who had had nephrosis and had received injections of acacia three weeks before death (× 160); (*b*) darkened areas attributable to positive stain for fat (× 160).

weeks before his death. Other patients had been given acacia for acute shock and had received from 30 to 60 Gm. of solution of acacia a few days before death. In all such cases, in spite of the considerable clinical quantities of acacia that had been administered, the livers were approximately of normal size; the surfaces were smooth, and the tissues were of normal consistency. Microscopic sections from these livers did not reveal the inner zonal and periportal lightly and clearly staining areas that were seen in the livers of the animals. The cytoplasm of the parenchymal cells contained fewer large vacuoles, and the stain was positive for fat (fig. 5). Acacia may have been present, but its presence was not definitive. The spleen and kidneys appeared normal.

Chemical Determinations of Acacia in the Liver, Spleen and Kidneys.—By chemical analysis, it appears that acacia is deposited in the liver, the spleen and the kidneys in that order in diminishing quantities. At the time when the first abdominal explorations were made and when the injections of acacia had been discontinued, the concentration of acacia in the serum was highest and acacia was being stored in the liver. Approximately ten weeks later, when the second abdominal explorations were made, the concentrations of acacia in the serum were decreasing, and storage of acacia in the livers of all animals appeared to be increased. The hepatic tissues analyzed at the time of the last exploration contained less acacia than at any other of the four periods, and the concentration of acacia in the serum was lowest. The data obtained indicated that by the time samples of tissue had been prepared for analysis glycogen had largely disappeared as an interfering factor. Qualitative colorimetric estimations with Bial's orcin test for pentoses supported this view.

In the animal that died, approximately 53.6 Gm. of acacia was accounted for in the liver, spleen and kidneys, 52 Gm. of which was in the liver. The acacia determined to be present in the liver of this animal represented about 18.8 per cent of the total quantity of acacia injected into the animal. Andersch and Gibson have reported that 50 per cent of acacia injected intravenously into rabbits can be accounted for in the liver and about 30 per cent of that injected into dogs.

Effects on the Blood, Other Than Concentration of Acacia.—In table 2 are recorded the results of chemical determinations of the blood made on the 3 observed animals. Many workers have noted decrease in the total protein, affecting both the albumin and the globulin fractions, following intravenous injection of solution of acacia. Dick and associates [10] noted that the concentration of total proteins of dogs which had been given injections of acacia was decreased but that the concentration returned to normal after administration had been discontinued. Heckel and associates [11] suggested two mechanisms for this diminution: (1) that the hepatic cells, engorged with acacia, are not able to produce the necessary

TABLE 2.—*Determinations on the Blood During and After Intravenous Injections of Solution of Acacia*

	Weight, Kg.			Protein Fractions: Serum Protein, Gm. per 100 Cc.			Serum Albumin, Gm. per 100 Cc.			Serum Globulin, Gm. per 100 Cc.			Fibrinogen, Gm. per 100 Cc.			Hemoglobin, Gm. per 100 Cc.			Hematocrit Reading, Cells per 100 Cc.		
	Animal			Animal			Animal			Animal			Animal			Animal			Animal		
Date	9	7	8	9	7	8	9	7	8	9	7	8	9	7	8	9	7	8	9	7	8
1/ 7/42	6.4	7.0	5.8	...	...	...	...	...	...	...	...	...	....	....	....	....	....	....	....	....	....
1/15/42	6.4	7.0	6.0	4.8	3.3	3.0	...	...	...	...	...	...	....	....	....	....	....	....	52.0	42.0	12.0
1/21/42	6.4	7.4	5.8	5.0	3.2	3.2	...	...	...	...	...	...	....	....	....	....	....	....	52.0	40.0	26.0
2/ 5/42	7.0	7.4	5.8	5.7	4.1	3.4	...	...	...	...	...	...	....	....	....	....	....	....	53.0	34.0	30.0
2/19/42	7.0	7.2	5.5	4.9	3.8	3.2	3.0	2.0	1.7	1.8	1.8	1.5	0.27	0.16	0.23	15.3	12.4	4.2	46.0	32.0	22.0
3/ 3/42	7.2	7.2	5.4	5.0	3.9	2.7	3.2	1.9	1.5	1.9	1.9	1.2	0.43	0.39	0.32	17.1	12.4	7.9	49.0	39.0	29.0
3/19/42*	7.2	7.2	5.4	4.9	3.5	3.0	2.9	2.2	2.3	2.0	1.3	0.7	0.30	0.24	0.25	16.0	12.0	6.8	49.0	37.0	26.0
4/16/42	7.4	7.2	5.2	5.6	4.8	3.8	3.4	2.6	2.2	2.2	2.2	1.7	0.32	0.42	0.58	16.8	12.2	10.0	54.0	43.0	37.0
4/30/42	7.8	7.2	4.8	5.7	5.4	4.6	3.7	2.9	2.8	2.0	2.5	1.9	0.46	0.34	0.46	17.9	14.6	9.5	56.0	50.0	34.0
5/29/42	5.6	7.4	5.6	5.5	5.2	5.3	3.4	2.6	3.1	2.1	2.6	2.2	0.53	0.48	0.76	15.3	12.0	11.4	53.0	47.0	40.0
9/ 8/42	6.6	6.6	...	6.1	4.7	...	3.7	3.2	...	2.4	1.5	...	0.41	0.43	....	8.5	7.4	....	54.0	42.6	....
11/30/42	6.8	6.4	...	5.9	4.9	...	2.8	1.8	...	3.1	3.1	...	0.61	....	....	12.0	15.2	....	56.0	46.0	....
2/ 4/43	6.8	6.6	...	6.1	5.5	...	3.5	3.0	...	2.6	2.6	...	0.52	0.46	....	16.1	15.3	....	56.0	52.0	....
4/21/43	...	...	...	5.8	5.1	...	3.6	3.0	...	2.2	2.1	...	0.27	0.28	....	....	....	....	48.0	44.0	....
3/23/44	6.6	7.2	...	6.1	5.3	...	3.7	3.4	...	2.4	1.9	...	0.47	1.00	....	16.0	16.0	....	54.0	56.0	....

* Acacia was not injected after 3/23/42.

10. Dick, M. W.; Warweg, E., and Andersch, M.: Acacia in the Treatment of Nephrosis, J. A. M. A. **105**:654-657 (Aug. 31) 1935.

11. Heckel, G. P.; Erickson, C. C.; Yuile, C. L., and Knutti, R. E.: Blood Plasma Proteins as Influenced by Intravenous Injection of Gum Acacia, J. Exper. Med. **67**:345-360 (March) 1938.

amount of plasma protein and (2) that the plasma protein is removed from the blood stream in an attempt to return plasma volume and colloidal osmotic pressure to normal limits. Yuile and Knutti [12] observed that the plasma volume is increased after injections of acacia and that while this increase obtains the concentration of total protein is low. Goudsmit and co-workers [13] reported that most of the decreases in concentration of total protein were due entirely to dilution. We do not have enough information definitely to explain the decrease in concentration of total protein. It may be a dilution effect to compensate for circulating serum acacia. However, we do not believe that it is caused by, but rather that it accompanies, the administration of acacia; that is, the function of the hepatic cells is not seriously disturbed. Also, as has been reported, some others of the functions of the hepatic cells are not disturbed by the presence of acacia.[14]

In a series of determinations made on normal animals, we have found the normal concentration of total proteins to range between 6.5 and 7 Gm. per hundred cubic centimeters of serum. The concentration of total proteins was decreased similarly in the blood of the 3 observed animals. The degree of decrease was proportional to the amount of solution of acacia given. In the blood of the 2 animals given the larger amounts of solution of acacia, the concentration of total protein was lowered to a greater degree than it was in the blood of the animal which received the smaller amount of solution of acacia. Also, this decrease was greater in the animal which received the most solution of acacia than it was in the animal which received the next to the largest amount. The decreases noted occurred practically at the beginning of the period of administration of acacia, and the values maintained themselves or tended to improve in spite of continued injections over the remaining portion of the period of seventy-six days of administration. The normal plasma albumin fraction is about 4 Gm. and the globulin fraction, about 3.5 Gm., per hundred cubic centimeters; the ratio is slightly greater than 1:1. In comparison with these normal values, it is suggested that the albumin was decreased more than the globulin and that also the ratio was reduced below that of normal animals; the ratio, however, was not inverted. Edema did not develop in 2 of the observed animals, in 1 of which the concentration of total protein was depressed below 4 Gm. per hundred cubic centimeters. The animal which received the most acacia, and the value for total protein of which was lowest, had ascites at the time when the injections of acacia were discontinued and when the first exploratory operation was performed. Also, there was slight edema of the hindlegs at that time. At no time was generalized edema present. In spite of the high concentration of acacia in the serum and the low value for total proteins, the dogs that were still living at the time this paper was written appeared alert and well, as has been said.

The total plasma proteins and also the albumin and globulin fractions began to increase almost at once after the injections of solution of acacia had been discontinued. In one month, the concentration of total proteins in the blood of all 3 observed animals increased from 0.7 to 1.3 Gm. per hundred cubic centimeters. This indicates that the serum proteins could be restored. The return was slow but steady; the values were not within the normal range at the end of the period of observation.

The ultimate recovery of a patient with hypoproteinemia is dependent on his ability to restore to normal the concentration of total proteins. Clinically, we have observed many patients with chronic glomerulonephritis and the nephrotic syndrome the concentration of whose total proteins has increased from below 4 Gm. per hundred cubic centimeters to normal—that is, from 3.6 to 6.2 and from 4 to 7.5 Gm., per hundred cubic centimeters—after therapeutic administration of acacia. This increase has taken place in eleven to eighteen months following treatment. One of these patients, aged 35 years, had been known to have renal disease for four years when first treated at the clinic. At the time of a recent follow-up inquiry, this patient stated that he felt well and was working full time daily. This is the evidence on which we base the belief that the temporary decrease in the concentration of total protein accompanies, but is not caused by, the administration of acacia.

Under the conditions of this study the fibrinogen was never depressed to the low limits reached in the work of Heckel and his associates and of Yuile and Knutti. The values for fibrinogen fluctuated, but not directly with the decrease in the total proteins nor with the decrease in the fractions of albumin and globulin. These determinations did not vary directly with the quantity of solution of acacia administered. Also, the

12. Yuile, C. L., and Knutti, R. E.: Blood Plasma Proteins as Influenced by Intravenous Injection of Gum Acacia, J. Exper. Med. **70**:605-613 (Dec.) 1939.

13. Goudsmit, A., Jr.; Binger, M. W., and Power, M. H.: Acacia in the Treatment of the Nephrotic Syndrome, Arch. Int. Med. **68**:701-712 (Oct.) 1941.

14. Metcalf, R. G., and Hawkins, W. B.: Plasma Protein, Bile Salt and Cholesterol Metabolism as Influenced by Multiple Injections of Gum Acacia in Bile Fistula Dogs, Am. J. Path. **15**:429-444 (July) 1939.

values for fibrinogen did not remain low during the period of administration of solution of acacia. One determination of plasma fibrinogen made on animal 7, which received the second largest amount of solution of acacia, reached 1 Gm.; this indicated that the source of supply of this protein fraction, which many workers feel is mainly the liver,[15] was able to sustain an increase of values for fibrinogen to above normal. If the plasma fibrinogen is used as a means of estimating the relative degree of hepatic function, according to these determinations the hepatic cells of the observed animals were not seriously injured. Other authors have reported that in determining concentration of fibrinogen they found the time necessary for the clot to form to be from twenty minutes to one hour. To determine whether precipitation time might be a factor in obtaining all the fibrinogen present in plasma that contained acacia, this process was controlled at twenty minutes, at one hour and overnight. The determinations of fibrinogen made at these different times on the different specimens of plasma were nearly alike.

The animal which received the largest dose of solution of acacia had a normal value for prothrombin during the middle part of the course of injections of acacia. Duplicate determinations on all 3 observed animals, sixty-nine days after the injections of acacia had been begun, also gave normal results. The coagulation time always was normal, between five and ten minutes. Clot retraction appeared normal. Bleeding time was normal.

The sulfobromophthalein sodium test of hepatic function was made fifty days after the injections of acacia had been begun, and it was found that less than 5 per cent of dye was retained by any of the 3 observed animals. These results were interpreted as being normal.

The concentration of hemoglobin also fluctuated, increasing slightly between injections, and, with reference to the 2 living animals, at the time of writing of this paper these values had returned to normal. Hematocrit values decreased after injections of solution of acacia and increased between injections. They became normal and continued so when the injections were discontinued (table 2). Macrocytic anemia has been observed repeatedly among patients whose hepatic substance has undergone varying degrees of damage and destruction.[16] The changes in the blood are variable, and anemia does not develop in all cases in which the liver has sustained damage. Blood smears taken in the early days of administration of acacia and throughout the period of observation, including the day on which administration was discontinued and two years following discontinuance of administration, were examined for evidence of macrocytosis. At all times the mean diameters of the cells were within the ranges determined on normal dogs, 6.5 to 7.2 microns.

There was definite increase in the sedimentation rate of the animals which received acacia as contrasted with that of normal dogs. The greatest increase was that of the animal which received the largest amount of acacia, and the least increase occurred in the animal which received the smallest amount of acacia.

SUMMARY

In a period of seventy-six days 3 dogs received eight, twenty-two and twenty-six times the amount of acacia usually given therapeutically to patients. The animal that received the largest amount died four months after discontinuation of the injections of acacia. No evidence of profound functional hepatic injury was found, although large amounts of acacia were present in the liver. In the livers of the other 2 dogs acacia was present twenty-six months after the last injection of acacia. These animals survived four exploratory laparotomies with biopsy of the liver and remained in good condition. These results do not contraindicate the use of acacia therapeutically under careful management.

15. Foster, D. P., and Whipple, G. H.: Blood Fibrin Studies: IV. Fibrin Values Influenced by Cell Injury, Inflammation, Intoxication, Liver Injury and the Eck Fistula; Notes Concerning the Origin of Fibrinogen in the Body, Am. J. Physiol. **58**:407-431 (Jan. 1) 1922. Berryman, G. H.; Bollman, J. L., and Mann, F. C.: The Influence of the Liver on the Proteins of the Blood Plasma, ibid. **139**:556-562 (Aug.) 1943.

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HABITUS OF PATIENTS WITH ACTIVE SICKLE CELL ANEMIA OF LONG DURATION

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NEW ORLEANS

In many disease states the general physical characteristics of the patient aid the examiner in making an etiologic diagnosis. In acromegaly, cretinism or myxedema, achrondroplasia, pituitary basophilism, mongolism, adiposogenital dystrophy, rickets and many other conditions valuable information can be obtained by observation of the nude subject. Patients with long-standing active sickle cell anemia have a fairly characteristic habitus [1] which may be considered typical of the disease. In several instances the diagnosis of sickle cell anemia was suspected on inspection of the nude subject by physicians familiar with the sickle cell anemia type of habitus. In these instances subsequent hanging drop preparations showing sickling and positive diagnostic parameters [2] indicated the correctness of these observations.

The purpose of this paper is to point out the characteristics of the habitus of patients with sickle cell anemia of long duration and to discuss some of the factors which contribute to its production.

Fifteen patients between the ages of 6 and 32 years with active sickle cell anemia and 4 patients between the ages of 30 and 47 years with sicklemia (the sickling phenomenon without anemia or other manifestations of the disease) were chosen for study. All patients with sickle cell anemia had sickling of the erythrocytes and positive diagnostic parameters. Reports on detailed anthropometric studies on these patients have been published elsewhere.[1] All patients were Negroes, and most of those with sickle cell anemia had been hospitalized and observed repeatedly during exacerbations of their disease. The majority of the patients had associated heart disease, which probably resulted from their long-standing anemia.[3] The adult and adolescent male patients had the most numerous hospital admissions, and as a group represented the most severely diseased patients. For each patient with sickle cell anemia, 10 control subjects of the same sex, age and color were chosen for comparison.

Two methods of study were utilized: (1) The general appearance of the subjects was evaluated by direct observation and from photographic prints, and (2) anthropometric measurements were obtained by means of standard anthropometric technic and equipment.[4] Of

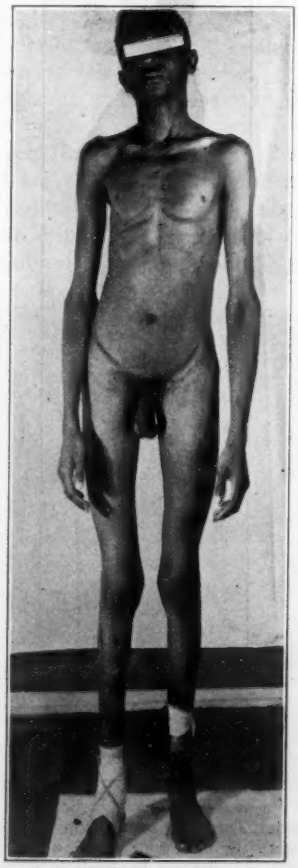

Fig. 1.—This 19 year old patient has had approximately 2,000,000 erythrocytes for at least the past three years. There is no evidence of cardiac disease. He illustrates the linear type of habitus and a definite loss in weight. The legs are long and the trunk relatively short.

From the Department of Medicine, Tulane University of Louisiana School of Medicine, and Charity Hospital.

1. Winsor, T., and Burch, G. E.: The Habitus of Patients with Sickle Cell Anemia, Human Biol. **16**: 99-113 (May) 1944.

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3. Winsor, T., and Burch, G. E.: Electrocardiogram and Cardiac State in Patients with Sickle Cell Anemia, to be published.

4. Hrdlička, A.: Practical Anthropometry, Philadelphia, Wistar Institute of Anatomy and Biology, 1939.

these two methods the former yielded valuable information which could not be adequately recorded by direct measurement. The latter gave significant information regarding in particular the skeletal structure.

THE HABITUS

Clinical Appearance.—The habitus of patients with active sickle cell anemia was often striking and at times characteristic, particularly of patients in whom the disease had been active for many years.

Fig. 2.—The legs are long and the trunk short. The anteroposterior diameter of the chest is increased as compared with the lateral diameter. The neck appears short. There is a definite kyphosis in the upper region of the thorax. The normal lower lumbar lordotic curvature is increased. The patient has complained of intermittent backache for the past four years. Roentgenographic changes in the bodies of the lumbar vertebrae are shown in figure 5.

The following characteristics were observed in the adult patients and are exemplified in figures 1 and 2.

The patients were slender and appeared relatively tall, although they were not necessarily of greater than average height. The shoulders and hips were narrow, producing a linear type of habitus (fig. 1). Occasionally the head was somewhat abnormal in shape (dolichocephalic, scaphocephalic or otherwise unusual). The neck appeared short. The normal upper dorsal kyphotic curvature was definitely accentuated (fig. 2), and the lower lumbar curvature was excessively lordotic. The arms and legs were thin and long and the trunk short (figs. 1 and 2). The chest was deep and narrow, producing a "hoop-chested" appearance. The hands, fingers, feet and toes were long and narrow. The hands have been well termed "spider hands" (fig. 3). Signs of hypogonadism (such as genital hypoplasia or atrophy, hypotrichosis and high-pitched voice) were occasionally encountered.

The appearance of the children was either slightly or strongly abnormal, depending on the

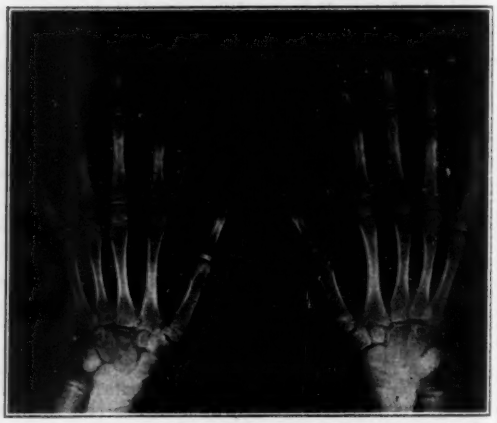

Fig. 3.—"Spider fingers" with generalized decalcification in a 17 year old girl who has had severe chronic active sickle cell anemia for at least eight years. Osseous changes are present elsewhere in the body.

severity and duration of the disease. In this group the thin arms and legs, the "hoop chest" and the protruding abdomen were the most outstanding characteristics (fig. 4). Any of the characteristics just described for the adult patients might be present, however.

In subjects with sicklemia no characteristic features were seen.

Roentgenographic Manifestations.—Although the roentgenographic observations were not constant, certain features should be mentioned. Changes in the skull were rarely present and consisted of decalcification with exaggeration of the normal reticulations, best seen in the region of the vertex. Only 1 of 8 patients for whom roentgenograms of the skull were taken showed the typical "hairbrush" appearance. The vertebral bodies sometimes showed pronounced de-

calcification and sometimes showed crescentic depressions of their superior and inferior borders (fig. 5). These changes were most noticeable in the lumbar region. The hands showed "spider fingers," with pronounced decalcification

Fig. 4.—This 7 year old patient demonstrates the deep chest, "hoop chest," and the protuberant abdomen. The spleen and liver were not palpable, and the abdomen was tympanitic. There has been no appreciable change in the degree of abdominal protuberance during the past three years.

of the metacarpal bones and phalanges (fig. 3) in some.

Anthropometric Measurements.—The mean, maximum and minimum measurements are listed in the table. All measurements were made according to the metric system, and the indexes are expressed in per cents. The average values for the patients with sickle cell anemia represent the averages for 3 male patients, the ages being 21, 24 and 30 years. The control groups consisted of 10 subjects each of the same sex, age and color, making a total of 30 control subjects.

The following points are emphasized in the table. The mean stature of the diseased subjects was slightly less than that of the controls. The mean weight of the patients with sickle cell anemia was noticeably less than that of controls (table *b*). Although both stature and weight were reduced, the weight was reduced more than the stature (table *c*). The span, like the stature, was less for the patients than for the controls; however, the relation of the span to the stature was not significantly different in the two groups (table *d*). The sitting height as compared to the stature was shorter in the diseased group than in the controls (table *e*). The average sitting height of patients with sickle cell anemia was 47.2 per cent of the stature, and of the control subjects it was 51.5 per cent. The average pubic height (measured from the floor to the superior portion of the symphysis pubis) was 55.9 per cent of the stature in the diseased group and 53.3 per cent in the control group. The average widths of the shoulders and pelvis were less in the diseased group than in the controls (table *f* and *g*). The pelvic width in the diseased

Fig. 5.—Changes in the vertebral bodies seen in the patient illustrated in figure 2. There is generalized decalcification with crescentic depressions of their superior and inferior aspects. The changes are most conspicuous in the fifth lumbar vertebra.

group was narrow in relation to the stature, being 14.6 per cent of the stature as compared with

15.9 per cent in the controls (table *h*). The circumferences both of the chest and of the abdomen were less for the patients than for the controls (table *i* and *j*).* The average abdominal circumference in the diseased group was relatively large, averaging 87.3 per cent of the thoracic circumference in the diseased patients and 83.5 per cent in the controls. The anteroposterior diameter of the chest averaged 77.5 per cent of the lateral diameter in the diseased group and 67.1 per cent in the controls. Items *m* and *n* in the table illustrate the long narrow character of the hand and foot. The circumference of the leg was decidedly reduced (table *o*). There was a tendency to dolichocephaly (table *p*).

Figures 6 and 7 show a few of the characteristics which constituted the drepanocytemic habitus in 7 children, 2 adolescents and 6 adults. These graphs illustrate the changes in body configuration which were observed at different age levels and may suggest changes which take place as growth and the disease processes progress. For each patient with sickle cell anemia, 10 normal subjects of the same sex, age and color were studied as controls, making a total of 70 children, 20 adolescents and 60 adults, or 150 control subjects in all. In the older age groups the patients were more linear in build than the controls (fig. 6). The circumference of the legs was considerably less than that of the legs of the controls at all age levels, and the difference was greatest in the adolescents and adults. The body weight was less than that of the controls. This difference also was greatest in the adults and adolescents. The hands were consistently longer and narrower than the hands of controls at all age levels (fig. 6). The sitting height was less than that of the controls in all three groups (fig. 7). The legs, from youth to adulthood, were somewhat longer than those of the

Mean Anthropometric Measurements for Three Patients with Sickle Cell Anemia and for Thirty Normal Subjects

Measurement or Index	Patients with Sickle Cell Anemia, Mean (Extremes) Cm., % or Kg.	Controls, Mean (Extremes) Cm., % or Kg.
(a) Stature	168.2 (171.0-165.5)*	171.8 (177.0-160.3)
(b) Weight	43.0 (45.0-41.0)	61.0 (79.5-51.2)
(c) Weight/stature	25.8 (26.3-24.8)	35.5 (44.8-41.9)
(d) Span/stature	105.9 (106.0-105.8)	106.2 (107.6-105.4)
(e) Sitting height/stature	47.2 (47.5-46.9)	51.5 (51.6-51.4)
(f) Shoulder width	36.5 (38.8-34.3)	41.0 (44.6-37.3)
(g) Pelvic width (intercristal)	24.6 (24.7-24.6)	27.4 (30.0-25.6)
(h) Pelvic width/stature	14.6 (14.9-14.4)	15.9 (16.9-15.0)
(i) Circumference of chest	72,5 (75.0-71.1)	86.3 (97.3-78.8)
(j) Abdominal circumference	63,3 (63.6-63.0)	72.1 (86.5-68.1)
(k) Anteroposterior diameter of chest	18.6 (19.0-18.2)	19.0 (21.9-17.2)
(l) Lateral diameter of chest	24.0 (26.0-22.0)	28.3 (31.7-24.4)
(m) Hand breadth/length	41.7 (43.1-40.8)	45.2 (45.6-44.1)
(n) Foot breadth/length	36.7 (37.0-34.2)	38.0 (41.3-37.2)
(o) Circumference of calf	22.1 (25.4-21.8)	32.5 (36.6-30.5)
(p) Head width/length	70.5 (72.7-68.2)	77.1 (78.5-76.1)

* Maximum and minimum values are shown within the parentheses.

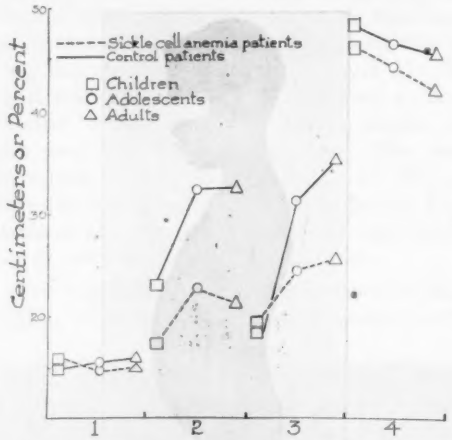

Fig. 6.—Changes in body configuration seen at three different age levels, during childhood, adolescence and adulthood. The figures represent the averages of 15 patients with sickle cell anemia and of 150 normal subjects used as controls. The numbers at the base of the graph represent the following comparisons: (1) width of the pelvis as compared with the stature in per cents; (2) circumference of the leg in centimeters; (3) body weight in relation to the stature in per cents, and (4) width of the hand in relation to the length in per cents.

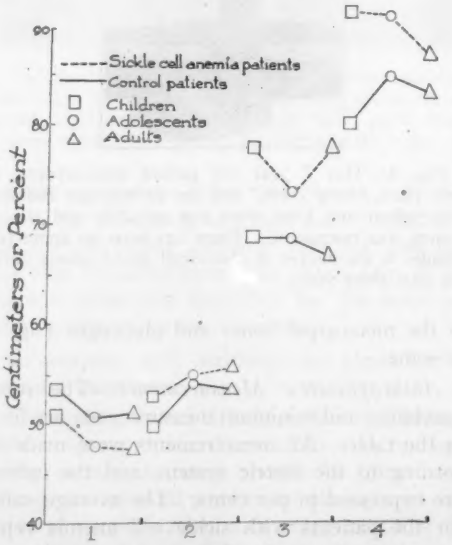

Fig. 7.—Other comparisons from the same patients described in figure 6 (all comparisons expressed in per cents): (1) sitting height in relation to the stature; (2) pubic height in relation to the stature; (3) depth of the chest in relation to the width, and (4) abdominal circumference in relation to thoracic circumference.

controls. The chest had a "hoop-chested" appearance in the three age groups. In the older age groups, the abdominal girth in relation to the thoracic girth was less than that of the controls (fig. 7).

Although in isolated instances the change in body form with increasing age was not distinctive, there was often a major difference between the body features existing in childhood and those seen in adulthood.

COMMENT

It is not surprising that the habitus is abnormal in patients with long-standing active sickle cell anemia, as practically all tissues of the body are affected by the disease processes. The organs involved are so numerous and the signs and symptoms so protean that Wintrobe has labeled this disease, in contrast to syphilis, the "little imitator." [5] The cardiovascular system is affected in about 90 per cent of patients, and a diagnosis of rheumatic or congenital heart disease was erroneously made for more than half the patients at Charity Hospital.[3] Involvement of the nervous system has produced paralytic changes, for which leg casts have been applied under the mistaken diagnosis of poliomyelitis. Pulmonary infarcts have been repeatedly and mistakenly diagnosed as bacterial or atypical pneumonia, and treatment for the former with sulfonamide drugs has produced definite injury to the kidneys, which were already injured by the disease process itself. The abdominal viscera, the spleen, liver and gastrointestinal tract, are all frequently involved; and erroneous preoperative diagnoses of ruptured peptic ulcer, ruptured gallbladder or acute appendicitis have led to unwarranted surgical intervention among these patients, in whom there is a high surgical mortality. In addition to the involvement of the aforementioned organs and organic systems, diseases of the endocrine, hemopoietic, vascular and osseous systems serve to produce changes in the body type. With involvement of so many organ systems, it is not surprising that the habitus of these patients is altered.

The anemia itself is probably of great importance in altering the habitus, as patients with sicklemia do not have an abnormal habitus.[1] Severe anemia produces poor nutrition to all organs and tissues, and the tissues most sensitive to anoxia will be affected to the greatest degree. If, as a result of anoxia, the gonads became atrophic and the pituitary remained active, a hypogonad type of habitus would result. The subject would be tall; the span would exceed the stature, and the bone age would be considerably retarded. If the gonads remained functioning normally and the pituitary activity was depressed, the subject would be of decreased stature; the span to stature ratio would not be abnormal, and there would be no noticeable retardation of the bone age. In most of the patients with sickle cell anemia described in this study, the stature was decreased, and in some there was also evidence of hypogonadism. Thus it is possible that both the gonadal and the pituitary activities were decreased to produce this picture.

At just what age characteristic features of the habitus manifest themselves depends to a great extent on the severity of the disease and on the age at which the illness becomes active. It is conceivable that the typical habitus might be present at or before birth if severe intrauterine sickle cell anemia were present. Definite deviations from the normal have been repeatedly recognized during the first decade of life of such patients.

The development of the sickle cell anemia habitus may be likened to the development of the habitus in patients with acromegaly. When the disease is far advanced and of long duration, the clinical picture is easily recognized. When the disease process is slight or when full physical development has not taken place, as in younger persons, the typical configuration is often not apparent. Also, like that of patients with acromegaly, the body type in sickle cell anemia is not due to genetic characteristics, as is sometimes true of patients, for example, with achondroplasia, but is a result of the disease processes themselves. The sickle cell anemia habitus is unlike the "peptic ulcer habitus" or the "poliomyelitis physiognomy." In these the habitus is said to be genetic in origin and allegedly indicates a "constitutional" predisposition to the disease.

The essential features of the sickle cell anemia habitus are as follows:

The adults have linear builds, emaciation, long legs, "hoop chests," short trunks, "spider hands," lumbar lordosis and upper dorsal kyphosis, tendencies to hypogonadism and general appearance of fragility. The children have "hoop chests," enlarged, protruding abdomens and thin legs. Changes encountered in adults may be present in children if the disease is severe.

At this point it is wise to draw attention to a few physical characteristics of normal American Negroes as compared with those of white Americans, as a knowledge of these physical traits helps to prevent confusion resulting from normal

5. Diggs, L. W.; Pulliam, H. N., and King, J. C.: The Bone Changes in Sickle Cell Anemia, South. M. J. 30:249-259 (March) 1937.

racial differences.[6] The adult Negro is allegedly shorter in stature and lighter in weight than the white adult. This may not be true of Negroes from all localities. They are more linear in habitus, and the head tends to dolichocephaly. The trunk is relatively short, and the pelvis is flat and narrow. The length of the hands and feet is great as compared with their widths. The arms and legs are long, and the span exceeds the stature by as much as 12 cm. in some Negro men. There is an increase in the curvature of the lumbar portion of the spine, which produces a relative lumbar lordosis. The physical characteristics of the normal Negro may be found elsewhere.[7] In sickle cell anemia these normal characteristics are often exaggerated.

The habitus of patients with sickle cell anemia superficially resembles that encountered in patients with primary hypogonadism. There are certain variations, however, which aid in differentiating these two conditions. In patients with sickle cell anemia the span does not tend to exceed 107 per cent of the stature. In primary hypogonadism the span may exceed 112 per cent of stature or more. In sickle cell anemia the stature is often less than normal, while in primary hypogonadism it is greater than normal. In sickle cell anemia "spider hands" are present. The fingers are long and thin, and there is roentgenologic evidence of decalcification of the metacarpals and phalanges. Localized areas of periostitis may be seen. The body weight in primary hypogonadism is not usually abnormally decreased, while in active sickle cell anemia it is usually sharply reduced. In both disease states the trunk is short as compared with the stature. In primary hypogonadism the decrease in the sitting height is due to definite overgrowth of the long bones of the legs, while in sickle cell anemia it is due for the most part to shortening of the trunk as a result of a decrease in the height of the lumbar vertebrae.[5] Unusual degrees of kyphosis and lordosis due to changes in the bodies of the vertebrae (fig. 3), enlargement of the abdomen, enlargement of the anteroposterior diameter of the chest ("hoop chest") and decreased circumference of the legs tend further to differentiate these two conditions.

In sickle cell anemia some of the adult patients with severe anemia have evidence of gonadal hypofunction. In men, the scanty facial, thoracic and pubic hair, the testicular atrophy and the high voice are striking features which are occasionally seen. Retarded bone age is present in some adult patients.[5] This was not encountered by us, however. The slight increase in length of the lower extremities over that seen in normal persons may be evidence of some degree of hypogonadism. This hypogonadism, when present, is a secondary and not a primary type.

Changes in the external and roentgenologic appearance of the bones of the skull have been discussed by others.[8] Scaphocephaly with roentgenologic evidence of thickening of the outer table of the vertex,[9] dolichocephaly[10] and macrocephaly[11] have all been described. Diggs, Pulliam and King[5] have pointed out that up to 1937 there were only 9 patients described in the literature in whom typical changes of the skull were present. In their series of patients, only 1 had significant osseous changes in the skull. The rarity of these changes should be appreciated. Of the 15 Charity Hospital patients in this series, only 2 had definite gross abnormalities in the shape of the skull. Of the 8 patients in whom the skull was studied roentgenographically, only 1 showed the "hairbrush" appearance with thickening of the outer table. It is felt that the so-called typical roentgenographic changes in the skull are both uncommon and nonspecific, as

6. Herskovits, M. J.; Cameron, U. K., and Smith, H.: The Physical Form of Mississippi Negroes, Am. J. Phys. Anthropol. **16**:193-201 (Oct.-Dec.) 1931. Hrdlička, A.: The Full-Blooded American Negro, ibid. **12**:15-33 (July-Sept.) 1928. Herskovits, M. J.: Anthropometry of the American Negro, New York Columbia University Press, 1930. Hrdlička, A.: Anthropology of the American Negro, Am. J. Phys. Anthropol. **10**:205-235 (April-June) 1927. Royster, L. T., and Hulvey, C. N.: Relations of Weight, Height, and Age in Negro Children, Am. J. Dis. Child. **38**:1222-1230 (Dec.) 1929.

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10. Rose, C. B.: Some Unusual X-Ray Findings in Skulls, Radiology **13**:508-514 (Dec.) 1929.

11. Grinnan, A. G.: Roentgenologic Bone Changes in Sickle Cell and Erythroblastic Anemia, Am. J. Roentgenol. **34**:297-309 (Sept.) 1935.

the changes cited are encountered in other clinical states, such as Cooley's anemia and congenital hemolytic icterus.[12]

It may be said that the habitus in patients with sickle cell anemia is a changing one and striking variations in the physical characteristics are to be expected. The dynamic character of the disease tends to mold and remold the body type as the extent and duration of the disease vary. Many patients with mild disease or with disease of short duration will not exhibit the habitus just described.

Sickle cell anemia presents a difficult problem in diagnosis, only because it is forgotten when a differential diagnosis is under consideration. One significant advantage of a knowledge of the habitus in active sickle cell anemia is that on inspection of the patient one is immediately reminded of the disease entity and thus has the disease in mind before considering its diagnosis.

SUMMARY

The habitus of 15 patients with sickle cell anemia and of 4 with sicklemia was studied.

The body type of patients with sickle cell anemia was variable in its manifestations because of the dynamic character of the disease. The presence of the sickle cell anemia habitus depends on the extent and duration of the disease processes and on the age of the patient at the time of onset. A characteristic habitus was not encountered in patients with sicklemia. The syndrome was most characteristic in adults and may be described as follows: The habitus was linear; the subjects were underweight; the hips and shoulders were narrow, and the stature was decreased. There was an increased upper dorsal kyphosis and lumbar lordosis and an increase in the anteroposterior diameter of the chest ("hoop chest"). The trunk was short and often showed roentgenologic evidence of flattening of the lumbar vertebrae. The arms and legs were long, but the relationship between the span and the stature was not abnormal for Negroes. The hands ("spider hands") and feet were long and narrow, and there was roentgenologic evidence of decalcification of the metacarpals and phalanges.

The external genitalia were occasionally atrophic, and facial hair was sometimes scanty.

In the children, many of the changes just mentioned were slight, the more constant observations being the "hoop chest," the large abdomen and the small circumference of the legs.

The factors which seem to contribute to the sickle cell anemia habitus are the anemia, the cardiovascular disease, the endocrine changes and the changes in the osseous system.

12. Williams, H. U.: Human Paleopathology, Arch. Path. **7**:839-902 (May) 1929. Diggs, Pulliam and King.[5]

CIRCULATION IN ACUTE MYOCARDIAL INFARCTION

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Myocardial infarction as a clinical entity differs in many respects from other forms of heart disease. In most other conditions leading to cardiac strain and insufficiency the abnormal process affects the heart slowly and gradually, allowing some time for adjustment and adaptation. In infarction the lesion in the myocardium develops suddenly, resulting in partial necrosis of it, and in surviving patients the remaining healthy muscle takes over the function of the whole unit instantaneously. The derangement of the circulation resulting from this sudden insult to the heart is well suited for clinical study, because the exact time of its onset is usually clinically evident. The clinical picture of myocardial infarction is well known. A great deal of work has been done on the clinical aspects, laboratory findings, course and prognosis of acute infarction, but only a few papers have dealt with the mechanism of circulatory adjustment following the attack. Experimental studies by Orias [1] and by Gross, Mendlowitz and Schauer [2] have thrown some light on the subject. Orias concluded that the adaptability of the portion of the cardiac muscle not affected by the experimental infarction is the most important factor determining the outcome. Gross and his collaborators found a decreased cardiac output and increased circulation time indicating left ventricular failure. Fishberg, Hitzig and King [3] studied circulatory dynamics in human beings with acute myocardial infarction and found two distinct mechanisms influencing the course of the disease: (*a*) shock and (*b*) heart failure. They felt that in some cases one mechanism predominates and in others the other one. A more recent study by Stead and Ebert [4] dealt with the same subject, and in it the authors emphasized the coexistence of both these factors in some of their cases. They expressed the opinion that both are manifestations of the same causative agent, which is diminished cardiac output following infarction. This cardiac, or "cardiogenic," mechanism of shock was discussed at length by Boyer [5] in a recent review. Grishman and Master [6] studied cardiac output by a physical formula and found it decreased after myocardial infarcts in 4 of their 5 cases. This was confirmed by Starr and Wood [7] who used the ballistocardiograph. Massie and Miller [8] found no changes in cardiac size by roentgenologic examination but noted signs of pulmonary congestion in most cases.

It is evident to all students of the subject that there are three distinct types of the disease, at least in the early stage following the acute attack: that of patients with predominant signs of severe shock; that of patients with dyspnea and pulmonary edema, often progressing into congestive failure, and that of patients without any symptoms and signs other than the initial attack of pain. While the first two types, being more dramatic, attract more attention in research, the third one is equally important from the practical viewpoint. Patients with severe shock or with cardiac failure show the highest mortality, and most of those who survive become cardiac invalids from chronic insufficiency. The milder, asymptomatic form of the disease is the one in which a high percentage of the patients recover. For these patients the attending physician faces a considerable responsibility, having to decide the length of rest in bed, the duration of con-

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2. Gross, L.; Mendlowitz, M., and Schauer, G.: Hemodynamic Studies in Experimental Coronary Occlusion, Am. Heart J. **13**:647 and 664, 1937; **14**:21 and 669, 1937; **16**:278, 1938.

3. Fishberg, A. M.; Hitzig, W. M., and King, F. H.: Circulatory Dynamics in Myocardial Infarction, Arch. Int. Med. **54**:997 (Dec.) 1934.

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7. Starr, I., and Wood, F. C.: Studies with Ballistocardiograph in Acute Cardiac Infarction and Chronic Angina Pectoris, Am. Heart J. **25**:81, 1943.

8. Massie, E., and Miller, W. C.: Heart Size and Pulmonary Findings in Acute Coronary Thrombosis, Am. J. M. Sc. **206**:353, 1943.

valescence and the completeness of occupational rehabilitation.

This study deals largely with the milder form of the disease in an attempt to observe the derangement of the circulatory dynamics in patients so affected. Of the two major phenomena of myocardial infarction, shock and cardiac insufficiency, the latter is more important from the long range point of view. Shock is an acute incident responsible for many early deaths, but it disappears completely if the patient is to survive. On the other hand, signs of cardiac insufficiency in the ordinary sense may persist and progress into a chronic stage, and their presence or absence will determine the degree of the patient's recovery. Thus, even if those who believe in the common origin and identical mechanism of shock and congestive failure are correct, it is obvious that shock ceases to play a part in the course of myocardial infarction after the first few days. The fact that myocardial infarction affects almost exclusively the left ventricle is well known, and it is not surprising, therefore, that signs of left ventricular insufficiency are the predominant clinical features in this disease. The most sensitive method of detecting early left ventricular failure is determination of the velocity of blood flow through the pulmonary circulation.

METHODS AND RESULTS

The subjects studied were 45 unselected patients with acute myocardial infarction. Patients were observed personally and were included only if the diagnosis was reasonably certain, if the infarction was thought to be very recent and if they survived for more than a week after admission to the hospital. Particular attention was given to clinical signs and symptoms of left ventricular insufficiency, namely, paroxysmal dyspnea, orthopnea, gallop rhythm, pulsus alternans, basal pulmonary rales, hydrothorax and accentuation of the second pulmonic sound. The arm to tongue circulation time was measured by the bile salts method: 5 cc. (or 3 cc. for women) of a 20 per cent solution of sodium dehydrocholate (Procholon)[9] was rapidly injected into the cubital vein, and the time from the beginning of the injection to the signal given by the subject on perception of bitter taste was recorded. Only determinations with a sharp and distinct endpoint were considered. Determinations of circulation time were repeated at frequent intervals during the patients' stay in the hospital, and they varied from two to eight recordings. In half the patients, serial recordings of vital capacity were also made.

Of the 45 patients in this series, 32 recovered clinically and left the hospital asymptomatic; 5 remained in congestive failure varying in degree, and 8 died. Only 2 patients showed definite signs of shock.

Table 1 summarizes the results. They are based on two sets of figures: the shortest circulation time recorded during the first week after the attack and the last recording made before the patient left the hospital. Values for circulation time were classified arbitrarily as normal (10 to 16 seconds), borderline (17 to 20 seconds), slightly prolonged (21 to 25 seconds), moderately prolonged (26 to 35 seconds) and definitely prolonged (over 35 seconds). Change in the circulation time was considered significant when it was 3 seconds or more in the first two classes and 5 or more in others. The table indicates that the initial reading was within normal limits in only 3 cases, with borderline values in 14, and was prolonged in 28. All patients who did not recover clinically showed prolonged circulation times at the time of their admission. Twenty-nine patients, including 24 out of the 32 with clinical recovery, showed shortening of the circulation time on subsequent determinations. Of the 13 remaining, 5 showed shortening of the circulation time coinciding with the initial improvement, but they died or had relapses later on. An additional analysis of the group of patients who recovered clinically showed that out of the 24 in which the circulation time became shorter with recovery, in 15 its final values were within normal limits, in 8 there were borderline readings and in 1 only did the circulation time remain above 20 seconds in spite of clinical recovery.

TABLE 1.—*Circulation Time in Relation to Clinical Result* *

Circulation Time	Recovered	Unimproved	Died	Total
Normal (10 to 16 seconds)	3 (1)	0	0	3 (1)
Borderline (17 to 20 seconds)	14 (10)	0	0	14 (10)
Slightly prolonged (21 to 25 seconds)	11 (9)	2 (1)	3 (0)	16 (10)
Moderately prolonged (26 to 35 seconds)	4 (4)	1 (0)	4 (1)	9 (5)
Definitely prolonged (Over 35 seconds)	0	2 (2)	1 (1)	3 (3)
Total	32 (24)	5 (3)	8 (2)	45 (29)

* The figure on the left is based on the shortest circulation time recorded during the first week after the attack; the figure in parentheses indicates shortening of the circulation time on subsequent determinations.

For 15 patients recordings of circulation time were made frequently enough to allow discovery of the approximate time after the attack when the final (or shortest) values were reached. These occurred: at the end of the first week in 2 patients, during the second week in 4 patients, during the third week in 4 patients and during the fourth week in 5 patients.

For 22 patients serial determinations of vital capacity were made. Because of the influence of many noncardiac factors on the vital capacity,

9. The E. R. Squibb Co. furnished a generous supply of Procholon for this study.

no normal values were set, and the method was used only to observe changes in the same person after a myocardial infarction. These changes were found to be related to changes in circulation time. Table 2 presents the initial and the final readings of vital capacity and of circulation time in the 15 patients out of the 22 who recovered. It shows that in 9 of them there was parallel improvement evidenced by both methods, the increase in vital capacity corresponding to shortening of circulation time; in 4 circulation time but not vital capacity improved, and in 2 some improvement of the vital capacity occurred, while the circulation time was unchanged.

The longest circulation time after a myocardial infarction was for some patients registered within twenty-four hours after the attack, while in others it occurred some days afterward. In 10 patients the determinations were spaced very closely in the first week of the disease, and it was found that in 6 the peak was reached in twenty-four hours, in 1 after forty-eight hours and in 3 three days after the attack.

TABLE 2.—*Comparative Changes in the Circulation Time and Vital Capacity at the Onset and at the End of the Period of Observation*

Patient	Initial Reading		Final Reading	
	Vital Capacity, Cc.	Circulation Time, Seconds	Vital Capacity, Cc.	Circulation Time, Seconds
J. N.	3,000	24	3,700	16
M. C.	2,000	26	2,300	15
R. B.	1,600	19	2,600	14
J. N.	3,200	25	3,200	16
J. C.	1,100	34	2,600	18
V. S.	3,500	20	3,500	17
R. T.	3,000	20	3,000	15
F. W.	1,700	15	2,000	14
J. C.	2,500	22	3,100	16
H. C.	3,400	19	3,400	14
T. H.	2,200	18	2,600	14
J. G.	1,100	16	1,100	16
F. H.	2,000	33	2,600	23
E. S.	2,700	18	3,100	17
C. C.	2,300	25	2,600	17
Average	2,350	22.3	2,750	16.1

All the patients were divided according to the presence or absence of clinical signs and symptoms of cardiac insufficiency. In the group in which these features were evident, the initial circulation times in seconds were as follows: 16, 20, 20, 21, 21, 22, 22, 25, 25, 25, 28, 31, 31, 33, 34, 35, 35, 42 and 50, being 19 cases in all, with an average circulation time of 28 seconds. In the remaining 26 cases, without any circulatory insufficiency, the results in seconds were: 15, 16, 17, 17, 17, 18, 19, 19, 20, 20, 20, 20, 20, 21, 21, 22, 22, 22, 24, 24, 25, 25, 26, 26 and 36, with an average of 21.4 seconds. Even though this figure is significantly smaller than the one in the first group, as it was expected, it is still more than 50 per cent higher than the normal average of 13 seconds.

COMMENT

It is generally accepted that measurement of the arm to tongue circulation time is a valuable method of estimating the velocity of blood flow through the pulmonary circulation. This is based on the knowledge that the time from the injection of the substance into the vein to its entrance to the right side of the heart and the time of its flow from the left side of the heart to the receptor organ are both very short, so that most of the "circulation time" in the usual sense is spent on the flow through the lesser circulation, particularly through the pulmonary capillaries. The circulation time consists of four segments: the three just named and the reaction time, which is the time from the moment the fastest flowing particles of the substance arrive in the receptor organ until a sufficient concentration of them accumulates to stimulate the organ. The reaction time is negligible in normal subjects with the use of the proper technic, namely, the fastest possible injection of the substance into the vein. The slower the velocity of the blood flow, the more diluted is the substance, requiring longer time to stimulate the end organ and thus causing an additional delay in the circulation time in pathologic cases.

Numerous methods of measuring the circulation time are being used. In all of them an indifferent foreign substance is injected into the vein. The arrival of the substance in the receptor organ is recognized (*a*) by the patient's subjectively perceiving an abnormal sensation (bitter or sweet taste or heat sensation), (*b*) by objective signs such as cough or sighing respiration and, finally, (*c*) by means of specially devised detectors, as for example, for radioactive substances or for fluorescein. Most of the methods used are fairly reliable, although errors occur occasionally, mostly due to an exaggeration of already definitely prolonged circulation times by dilution of the substance. The recent reports by Lilienfeld and Berliner and Esser and Berliner [10] demonstrating discrepancies of duplicate readings of circulation time are not confirmed, particularly for normal or for slightly prolonged readings.

10. Lilienfeld, A., and Berliner, K.: Duplicate Measurements of Circulation Time Made with Alpha Lobeline Method, Arch. Int. Med. **69**:739 (May) 1942. Esser, K. H., and Berliner, K.: Duplicate Measurements of Circulation Time with the Saccharine Method, Ann. Int. Med. **19**:64, 1943.

One of the methods they used is shown to be unreliable (alpha lobeline hydrochloride).[11]

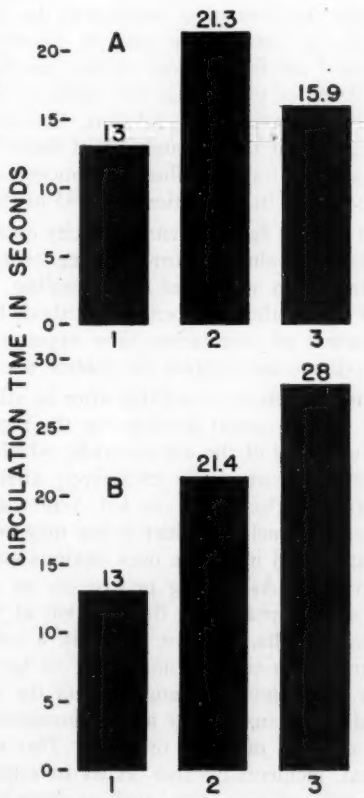
Determination of circulation time is thus a generally accepted clinical method of measuring the velocity of the blood flow through the pulmonary circulation and may be considered accurate and reliable when used judiciously. It appears, however, that its accuracy decreases with the lengthening of the circulation time when to the low velocity of blood flow due to cardiac insufficiency other factors are added. These factors not only lengthen unduly the time but also introduce a variable element obscuring the relation of circulation time to cardiac function and to the clinical course. For instance, when 2 patients with cardiac insufficiency show circulation times of 30 and 60 seconds, it is doubted whether in the second patient the velocity of the blood flow is actually slowed twice as much as in the first.

In this study most of the patients had only slightly or moderately prolonged circulation times, which are in the range of the sensitivity and reliability of the method. Therefore the statistical significance and accuracy of the data appear unquestionable.

It appears from the results presented that in early stages of acute myocardial infarction there is a definite increase in circulation time, which means slowing of the pulmonary circulation. This fact indicates that left ventricular insufficiency is the rule in almost all cases, even though it is clinically apparent in only a few. As healing of the infarct takes place, the cardiac function improves, and the circulation time usually returns to normal or to borderline values. This is presented graphically in the chart (*A*). The chart (*B*) also presents the values for circulation time of patients with clinically evident left ventricular failure contrasted with those of patients without symptoms of cardiac insufficiency. The fact that in asymptomatic patients the value is decidedly increased, though shorter than in the first group, is the most important finding of this study. It fits in with the result of roentgenographic demonstration of latent left ventricular failure in cardiac infarction by Massie and Miller.[8] Roentgenologic demonstration of left ventricular insufficiency by presence of pulmonary congestion, though helpful, is a less constant and less sensitive measure than the circulation time.

11. Hussey, H. H.; Cyr, D. P., and Katz, S.: The Comparative Value of Calcium Gluconate, Magnesium Sulfate and Alpha-Lobeline Hydrochloride as Agent for Measurements of Arm-to-Tongue Circulation Time in Fifty Patients With and Fifty Patients Without Heart Failure, Ann. Int. Med. **17**:849, 1942.

The absolute values of individual results are somewhat diminished by the presence of apparently insignificant delays in the circulation in some cases, even though the statistical value of the group as a whole is obvious. The presence of so many borderline readings can be explained first of all by the overlapping of normal and pathologic results in individual cases. For example, if a patient with a normally high velocity blood flow (around 10 seconds) develops a myocardial infarction, his circulation may slow by 60 per cent without bringing the reading outside



A. Average circulation time: (1) normal value, (2) initial value in patients who recovered from myocardial infarction and (3) final value in the same group of patients.

B. Average circulation time: (1) in normal persons, (2) in patients without clinical signs and symptoms of left ventricular failure after myocardial infarction and (3) in those with obvious left ventricular failure.

the normal limits of 16 seconds. Difficulty in evaluating normal results because of wide individual variations occurs in many other clinical methods. This is exemplified in the data on vital capacity, for which no normal standards were set at all. This method could be used only

for studying the same person, and comparison of the readings in different persons is of no value at all.

To this factor of overlapping of normal and abnormal values another one may be added which also tends to divert the average circulation time toward normal, and that is the possible effect of noncardiac factors on the velocity of the blood. It is well known that in hypothyroidism and polycythemia the circulation is slowed, while in anemia and hyperthyroidism velocity is increased. Other causes of shortened circulation time are fever, exercise and excitement with tachycardia. The effect of fever or of excitement due to pain may play a considerable part in counteracting the slowed circulation from cardiac insufficiency. This series of patients is too small to demonstrate conclusively this relation, but one can speculate about the possibility that these factors could account for the failure to demonstrate significant delays in circulation in all 45 of them.

The use of data on vital capacity proved of considerable value in corroborating changes in the circulation connected with healing of the infarct by another independent method, but determination of circulation time appears to be more reliable and simpler for routine use.

Thus it is demonstrated that after an infarction of the heart a strain develops on the remaining healthy muscle of the left ventricle, which is the part of the heart almost exclusively affected by infarction. The result is left ventricular insufficiency, which in milder forms may be slight and latent and in severe ones obvious and even irreversible. As healing progresses, an adjustment and adaptation of the function of the left ventricle results, in most cases, in a return of the circulation time to normal or to borderline values. The initial derangement of the circulation after the insult may appear immediately or after a period of hours or days. This variable interval, incidentally, also occurs in some other phenomena of infarction, such as drop in blood pressure.

It is felt that the result of this study is of more than academic interest. It may be applied in selected cases as an aid in diagnosis and prognosis of myocardial infarction. The observation that the circulation time is prolonged in myocardial infarction may help to differentiate this from other conditions associated with similar attacks of pain in the chest. This differential diagnosis is often difficult, especially when the electrocardiogram is equivocal, as in atypical infarcts or in those associated with bundle branch block. In some such cases it may be difficult to decide whether the pain originates in the heart or in other organs and, if in the heart, whether it is due to an infarction of the myocardium, to transient coronary insufficiency or to pericarditis. In many patients a determination of the circulation time may offer an important clue, particularly when the reading is either definitely prolonged or very short. For instance, if the diagnosis is in doubt, a circulation time of 24 seconds would make the diagnosis of infarction very likely, as in no other condition associated with pain in the chest is there a cause for slowing of the pulmonary circulation in the absence of obvious heart failure. On the other hand, in a similar case with a circulation time of 11 seconds other diagnoses than cardiac infarction should be considered, especially when fever and tachycardia are absent.

Recent studies by Barnes and Burchell [12] and by Wolff [13] dealing with acute pericarditis have shown the difficulty of distinguishing this condition from myocardial infarction. Here the value of the determination of circulation time in the differential diagnosis may be of particular interest.

While a single determination of the circulation time is useful in the differential diagnosis of myocardial infarction, serial readings may help determine the progress of the patient recovering from it. In patients who after the initial attack of pain present no signs and symptoms there is no clinical indication as to the satisfactory healing of the lesion. Laboratory procedures are often not helpful and may even be misleading. For instance, electrocardiographic evidence of changes may persist for months or even become permanent, regardless of the clinical condition of the patient. Fever and leukocytosis last only a short time. The sedimentation rate may be increased for many weeks after the patient is apparently well, but this persistence does not necessarily indicate deficient healing. Therefore, study of the circulatory readjustment after a myocardial infarction offers a method of observation of the convalescing patient by estimation of the most important single factor in recovery: the function of the left ventricle. The method has the great advantage of being simple, safe and inexpensive and does not require any special equipment. It is logical to assume that a patient with a more noticeable initial slowing of the circulation time and a slower rate of accelera-

12. Barnes, A. R., and Burchell, H. B.: Acute Pericarditis Simulating Acute Coronary Occlusion: Report of Fourteen Cases, Am. Heart J. **23**:247, 1942.

13. Wolff, L.: Acute Pericarditis Simulating Myocardial Infarction, New England J. Med. **230**:422, 1944.

tion ought to be hospitalized longer than the one who shows a mildly prolonged circulation time which returns to normal in a few days.

SUMMARY

Forty-five unselected patients with acute myocardial infarction were studied by serial determination of the arm to tongue circulation time and 22 of them also by serial determinations of vital capacity. The results were correlated with the clinical observations and with the course of the disease.

In all but 3 patients the initial readings of the circulation time were above normal, the average value for the series being 24.3 seconds (normal 13 seconds). In 24 of the 32 patients who recovered clinically there was a significant decrease in the circulation time on subsequent readings.

Prolonged circulation time indicating left ventricular failure was found most definitely in patients with clinical evidence of cardiac insufficiency (19 patients with an average value of 28 seconds); however, in patients who were entirely asymptomatic except for pain there was still a definite delay, indicating latent left ventricular failure (26 patients with an average of 21.4 seconds).

Determinations of circulation time are of practical value and are thought to be worth while as a routine procedure in myocardial infarction (*a*) as an aid in diagnosis by helping to differentiate it from other conditions associated with pain in the chest and (*b*) in evaluating the progress of circulatory readjustment after an infarction by means of repeated determinations of circulation time.

Correspondence

PERNICIOUS ANEMIA AND RELATED MACROCYTIC ANEMIAS

To the Editor:—I should like to present the following concept of pernicious anemia and related macrocytic anemias for the consideration of your readers.

Just as pellagra is no longer considered a "disease" of the skin, gastrointestinal tract or nervous system but is classified rather as a vitamin deficiency disorder, manifested by a disturbance in the metabolism of probably every tissue cell, so, too, the known facts relating to pernicious anemia may be construed to define not a "blood disorder" but one which affects the metabolism of diverse systems of the organism.

Thus, the "intrinsic factor," possibly an enzyme secreted into the stomach (Lasch [1937], Taylor [1938], Gessler [1940]), acts on the "extrinsic factor," possibly some portion of the vitamin B complex (Goodall [1932], Ungley [1933 and 1934], Strauss [1932], Wintrobe [1939]), with the resultant formation of a thermolabile substance which is conveyed to the liver by way of the blood stream. The liver then transforms this thermolabile substance into a thermostable compound, namely, "liver extract principle." Thus the liver functions not merely as a storage organ but as an active participant in the elaboration of liver extract principle.

I am also suggesting that "liver extract principle" is in reality a vitamin, which is possibly concerned with the proper functioning of some enzyme system, in a manner similar, perhaps, to that of nicotinamide, which is involved in enzyme reactions by way of coenzymes I and II (diphosphopyridine and triphosphopyridine nucleotides).

If such a function is conceded for "liver extract principle," the widespread disturbances of pernicious anemia — the gastrointestinal, the nervous, even the blood picture itself — then become readily explainable on the basis of a disordered metabolism involving one or more particular enzyme systems with which "liver extract principle" is intimately concerned.

If the "intrinsic factor" is absent or deficient, as occurs in so-called true Addisonian pernicious anemia and in high gastric resection (Bence [1934], Goodman [1935]), no thermolabile factor can be formed and deficiency of "liver extract principle" results.

If the "extrinsic factor" is lacking, as may occur in certain instances of macrocytic anemia, again no thermolabile factor is produced and deficiency of "liver extract principle" results.

If the thermolabile factor is formed, by interaction of "intrinsic" and "extrinsic" factors, but is not absorbed, owing to increased intestinal impermeability, as may occur in sprue, or owing to gastrointestinal short circuits, the thermostable factor cannot be formed and again a deficiency of "liver extract principle" results.

If the thermolabile factor is absorbed and conveyed by the blood stream to the liver, thermostable "liver extract principle" can result only in the presence of a liver which is functionally adequate to elaborate "liver extract principle." It is possible that in those instances in which macrocytic anemia, associated with other bodily disorders, occurs in the presence of hepatic damage, as in certain cases of hepatic cirrhosis, this function of the liver is disturbed and deficiency of the thermostable principle results.

Recent work indicates that the active principle of liver will soon be isolated and its structure determined. And with this the syndrome of macrocytic anemia will be removed from the category of "blood diseases" and added to the list of vitamin deficiency disorders.

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Book Reviews

Hypertension and Hypertensive Disease. By William Goldring, M.D., and Herbert Chasis, M.D. Price, $3.50. Pp. 253. New York: Commonwealth Fund, 1944.

The authors are well qualified to write a book on hypertension and hypertensive disease. They possess abundant clinical experience and are thoroughly familiar with the fundamental research work which has contributed so greatly to the recent advances in the study of hypertension.

During the past ten years, the progress made in the knowledge of hypertension and especially of hypertensive disease has resulted from a combined study of clinical work and laboratory research. The newer methods employed to investigate the changes both in renal function and in renal hemodynamics are intricate and may be confusing to any one unacquainted with them. However, the authors have explained these newer procedures in an adequate and simple manner.

The main part of the book deals with the evolution of the functional changes in the course of hypertension and hypertensive disease. The authors' concept of the genesis of hypertension, with special reference to the role of the kidneys, is given. From their investigations, they point out that the relationship between ischemia of the kidneys and hypertension is not the simple and direct cause and effect relationship that one would believe from experimentation with animals. Their conclusions are based not on research on animals but on a study of hypertensive persons. They believe that renal ischemia is one of the sequelae of hypertension and not a cause.

The renal genesis of hypertension in man can be accepted only in cases in which a pathologic process, such as diffuse glomerulonephritis, polycystic renal disease or atrophic pyelonephritis, involves both kidneys.

In their study of hypertensive patients, they used quantitative measurements of effective renal blood flow, filtration rate and maximal tubular excretory capacity. The earliest changes that occur in renal function and in renal hemodynamics are reduction in the maximal tubular excretory capacity, an associated reduction in renal blood flow and an increase in the filtration fraction. Such functional tests as those of urea clearance and of excretion of phenolsulfonphthalein are not altered in this early phase.

Changes that occur in the heart, the brain, the eyegrounds and the kidney are taken up and evaluated from the clinical standpoint.

A satisfactory amount of space is devoted to treatment. For any one looking for the authors' favorite prescription or method of treatment, there will be disappointment. Treatment is divided into medical, surgical and special measures. The authors' attitude from the therapeutic standpoint is rather nihilistic but probably deservedly so. They have no confidence in potassium thiocyanate as a therapeutic drug, opposing it chiefly on the grounds of the serious toxic complications which may develop. They believe that surgical operations, such as sympathectomy and similar procedures, have little or nothing to offer. They do, however, emphasize the importance of and the good results obtained from use of psychotherapeutic measures. The value of renal extract is believed to be uncertain.

This book is well written and easy to read. An intern, surgeon, urologist or any other person interested in hypertension will find an excellent discussion of the subject in this book. In the reviewer's opinion, the lessons contained in the book confirm the idea that the clinical study of hypertension cannot be separated from the more elaborate investigations carried out in the laboratory. Both must be integrated for a complete study of the disorder.

This is the first book of its kind which integrates the clinical and investigative phases of hypertension, and it is an important and excellent contribution to the subject.

Trichinosis. By Sylvester E. Gould, M.D. Price, $5. Pp. 322, with 128 illustrations. Springfield, Ill.: Charles C Thomas, Publisher, 1945.

The relative frequency of infestation of the adult population with Trichinella spiralis has been readily demonstrated by postmortem surveys. The relatively infrequent incidence of clinical trichinosis must result, therefore, from our failure to recognize sporadic cases, as it can manifest itself frequently with unusual symptomatology.

A complete treatise on this disease is a valuable contribution to current medical literature. This is a well written work embodying several interesting features, including historical aspects, parasitology, pathology, epidemiology, clinical aspects, treatment and control measures. One must call special attention to the section on the morphology of Trichinella spiralis, which includes several excellent illustrations and magnifications of microscopic studies.

In the section on epidemiology stress is placed on the pernicious practice of feeding pigs uncooked garbage containing scraps of raw trichinous pork, thus maintaining the pig as a reservoir for further human infection. The cycle then continues when uncooked or undercooked trichinous pork or pork products are eaten.

In his chapter on control measures, the author outlines how this cycle of transmission of infection might be attacked at several vulnerable points: first, by not permitting the feeding of swine with uncooked garbage; then, by encouraging proper inspection of pork and pork products, and, finally, by educating people to the fact that pork which is pink has not been sufficiently cooked and that pork which is not thoroughly cooked should not be eaten.

In the section on symptomatology it is pointed out that the varied clinical picture of trichinosis is only a reflection of the widespread distribution of parasites in the body, although generally the phases of the disease may be classified as the intestinal stage, the stage of muscular invasion and the stage of convalescence. Not infrequently the onset may be respiratory in nature, with coryza, cough and dyspnea. Neurologic symptoms which are common are supraorbital headache, vertigo, tinnitus and insomnia. Related to the involvement of the cardiovascular system is generally myocarditis, which may appear as early as the second week. This is probably on a toxic basis, since the larvae of Trichinella spiralis have never been found to be encysted within the heart muscle. The symptoms are substernal or precordal pain, tightness in the chest, shortness of

breath and palpitation. An important differentiation must be made between the edema of myocardial failure, which appears between the fourth and the eighth week, and the edema of the muscles of inflammatory origin, which will have begun to subside or will have disappeared by that time.

This book, complete in every detail, remains readable throughout. It includes a comprehensive bibliography, which enhances its value as a reference work.

Hipertensión arterial nefrógena. By Eduardo Braun-Menéndez and associates. Price, not given. Pp. 475, with 93 illustrations. Buenos Aires, Argentina: Librería y Editorial "El Ateneo," 1943.

In this book the authors give a review of their own experimental investigation and clinical experience, a digest of most of the modern works of other investigators and a comprehensive picture of the whole problem of arterial hypertension. Through their own experiences, they are well prepared to write a book such as this. The main part of the book deals with the genesis of renal arterial hypertension in man and in animals. The writers conclude that hypertension in man is not identical with the hypertension found in the experimental animal, and they emphasize the anatomic differences between the two forms.

Considerable space is devoted to the results of their pioneer investigations on renin, hypertensinogen and hypertensin. In brief, they hold to the theory that renin plus hypertensinogen produces hypertensin (angiotonin), which is responsible for the arteriolar constricting that produces hypertension. They discuss the enzyme hypertensinase, a substance from the kidney, which destroys hypertensin and allays arteriolar constriction. A careful account is given of the therapeutic possibilities of their own renal extract containing hypertensinase, and of the extracts of others, such as that of Page.

A special section of the book is given over to the etiology, clinical features and treatment of renal arterial hypertension in man. The action of various drugs, extracts and other agents used to reduce blood pressure is carefully and satisfactorily discussed.

Surgical procedures, such as removal of tumors of the kidney and correction of other conditions of the kidney, as well as operations on the adrenal gland and on the sympathetic nervous system, are taken up. Like most other authors, they believe that operations on the sympathetic nervous system for the control of hypertension have limited possibilities.

In this book there is a wealth of information on various problems of hypertension as well as a rich bibliography. It is well written, and there are many valuable illustrations and charts and an excellent page of colored photomicrographs. This is an excellent book, but it should be of more value to the investigator of the problem of hypertension than to the general practitioner.

Familial Susceptibility to Tuberculosis: Its Importance as a Public Health Problem. By Dr. Ruth Rice Puffer. Price, $2. Pp. 106, with 9 figures and 24 tables. Cambridge, Mass.: Harvard University Press, 1944.

This monograph stresses the importance of familial susceptibility in the development of active tuberculosis. The conclusions are reached by statistical analysis, and much of the material dealt with has been accumulated through the Williamson County Tuberculosis Study. This project was undertaken in Williamson County, Tenn., in 1931, with the assistance of the International Health Division of Rockefeller Foundation.

It is stated that more persons from families susceptible to tuberculosis appear to contract the disease than do persons from resistant families and that they have a more severe form. The suggestion is made that familial aggregation of tuberculosis has remained relatively stationary and that the decline in the tuberculosis death rate is due to a reduction in the proportion of tuberculous families in the population. There are interesting observations on the tuberculosis attack rate in children of tuberculous parents. This rate was high up to age 30 when the parent was known to have organisms in the sputum. In the children of tuberculous parents not known to have organisms in the sputum, active disease developed later, and by the age of 50 total attack rate in the two groups was equal. This would imply that with less exposure to tubercle bacilli the susceptible person contracts the disease later in life. The continued decrease in the incidence of tuberculosis might account for the relatively greater mortality rate of this disease in older age groups which has been observed in recent years.

In the chapter on the control of tuberculosis it is recommended that, in addition to the examination of the household contacts of persons with tuberculosis, the parents, siblings and children not in the household be followed and examined periodically. There is some question as to how feasible this would be with members of families widely scattered. An explanation of the statistical methods used is included in appendix B. The book can be read with profit by any physician interested in the problems of control of tuberculosis.

The Doctor's Job. By Carl Binger, M.D. Price, $3. Pp. 243. New York: W. W. Norton & Company, Inc., 1945.

Perhaps the keynote of Dr. Binger's book is to be found in the introduction when he says, "I am setting down these thoughts now because I feel the need of doing so." In other words, this is a simple, straightforward interpretation of medicine and its problems as one man sees them. Dr. Binger writes "on his own." He represents no foundation, forum, group or panel. He speaks for no organized interests of any sort. There are no political angles to his book. None the less, all the problems of medicine, so much under debate at the present time, are discussed in a sympathetic, well reasoned manner. Dr. Binger talks about general practice and specialization and conceives that some one must again take up the burden once borne by the family doctor. This man will not do everything himself but will "route" the patient to the proper specialists and finally will synthesize and interpret the whole situation. There are chapters on various disorders, usually with some emphasis on the psychosomatic aspects, and one finds discussions on forms of practice, socialized medicine and so forth.

In these days when the language is likely to be pretty badly abused in medical writing, Dr. Binger's style is a delight. Clearness, force and precision—the three graces of rhetoric—are set off by those decorations of language which, if properly used, add so much to the reader's pleasure—the apt quotation, the crisp aphorism, the *mot juste*. From the literary standpoint alone this book can be enjoyed equally by physician and layman.